Humboldt Universität zu Berlin

Dissertation

Prospects for the beneficial use of arbuscular mycorrhizal fungi in horticulture in combination with organic and inorganic fertilizers

Zur Erlangung des akademischen Grades

doctor rerum agriculturarum
(Dr. rer. agr.)

eingereicht an der
Landwirtschaftlich-Gärtnerischen Fakultät
der Humboldt-Universität zu Berlin
von

Dipl. agr. biol. Henrike Perner
19.08.1975 Jülich

Präsident der Humboldt-Universität zu Berlin
Prof. Dr. Christoph Markschies

Dekan der Landwirtschaftlich-Gärtnerischen Fakultät
Prof. Dr. Dr. h.c. Otto Kaufmann

Gutachter:
1. Prof. Dr. Eckhard George
2. Prof. Dr. Christof Engels
Tag der mündlichen Prüfung: 29.08.2006

Abstract

Arbuscular mycorrhizal (AM) fungi can be beneficial for horticultural crops due to their nutrient acquisition properties and stimulation of the plant metabolism. The present work focuses on the prospects of AM fungi a) to solve plant nutritional problems, b) to induce flower development of ornamental plants, and c) to improve the health potential of crop plants for humans.

Contribution of AM fungi to plant nutritional problems were investigated with leek, pelargonium and poinsettia plants on peat-based substrates with 20% and 40% compost additions. Moreover, lettuce plants were supplied on peat-based substrates with substrate own P, rock phosphate, or highly soluble P. Bunching onion and chinese chive were propagated on perlite in nutrient solution with low, medium and high NH_4^+:NO_3^- ratios. Mycorrhizal colonization, dry weight, and N, P, K, S, NO_3^-, Mg and Zn concentrations in plants were measured.

Mycorrhizal effects on bud and flower development of pelargonium and poinsettia plants were investigated on peat-based compost substrates.

Treatment effects on secondary metabolites in bunching onion and chinese chive were determined by exposing mycorrhizal and non mycorrhizal plants to three NH_4^+:NO_3^- supply ratios. The metabolites measured were glucose, fructose, and sucrose, total soluble solids, and organosulfur compounds (measured as pyruvic acid).

Colonization improved plant nutrient status and flower development. Under the described experimental conditions, however, plants did not consistently benefit in growth or plant composition from the mycorrhizal symbiosis. Additions of compost were a means of improving the substrate quality for an increased plant nutrient acquisition and plant growth in organic horticulture. The plant quality of *Allium* species in respect to organosulfur compounds was increased by taking the individual *Allium* species into consideration, their specific requirements for an optimal NH_4^+:NO_3^- supply ratio, and a possible AM effect on plant growth.

Keywords

Allium spec., ammonium, arbucular mycorrhizal fungi, compost, flower development, lettuce, nitrate, organic horticulture, organosulfur compounds, peat, pelargonium, poinsettia, rock phosphate

Kurzfassung

Aufgrund seines Nährstoffaneignungsvermögens und Stimulierung des Pflanzenmetabolismus kann der arbuskuläre Mykorrhiza (AM) Pilz im Gartenbau nutzbringend eingesetzt werden. Der Fokus der Arbeit liegt auf den Möglichkeiten des AM Pilzes a) pflanzenernährerische Probleme zu lösen, b) die Blütenbildung bei Zierpflanzen zu steigern und c) das Gesundheitspotential von Gemüse für den Menschen zu erhöhen (sekundäre Pflanzenmetaboliten).

Zur Lösung pflanzenernährerischer Probleme wurden Porree, Pelargonie und Poinsettie auf Torf-Substraten mit 20% und 40% Kompostzusatz untersucht. Ferner wurde Salat auf Torf-Substrat mit drei P Behandlungen getestet: substrateigenes P, Rohphosphat und lösliches P. Frühlingszwiebeln und Schnittknoblauch wurden in Nährlösungen auf Perlit mit niedrigem, mittlerem und hohem NH_4^+/NO_3^- Verhältnis ernährt. Gemessen wurde die AM Kolonisation, die Trockenmasse und die N, P, K, S, NO_3^-, Mg und Zn Konzentrationen im Spross.

Die Blütenbildung von Pelargonien und Poinsettien wurde auf Torf-Kompost-Substraten untersucht.

Der Einfluss auf die sekundäre Metaboliten von Frühlingszwiebeln und Schnittknoblauch wurde zusammen mit drei NH_4^+/NO_3^- Verhältnissen geprüft (s.o.). Untersucht wurden Glukose, Fruktose, Saccharose, lösliche Feststoffe und organische Schwefelverbindungen (gemessen als Pyruvat).

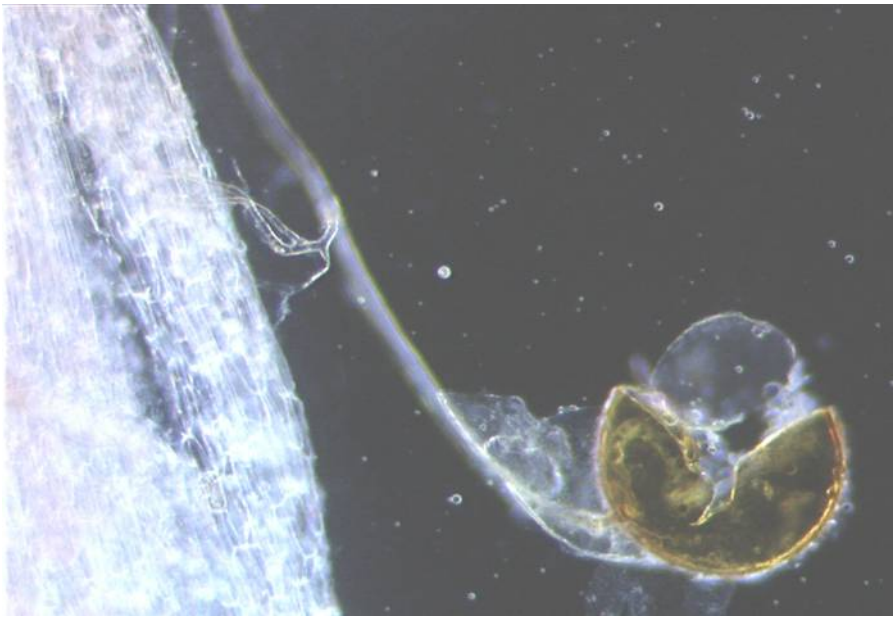
Eine AM Kolonisation konnte die Nährstoffversorgung der Pflanze verbessern und die Blütenbildung erhöhen. Jedoch profitierten die Pflanzen unter den beschriebenen experimentellen Bedingungen nicht durchgängig in ihrem Wachstum und Metaboliten vom AM Pilz. Die Zugaben von Kompost ermöglichte die Verbesserung der Substratqualität für die Nährstoffversorgung und das Pflanzenwachstum unter ökologischen Gartenbaubedingungen. Der Ertrag von gesundheitsfördernden organischen Schwefelverbindungen konnte in Abhängigkeit von der jeweiligen *Allium* Spezies, durch eine Variation des Ammonium/Nitrat Verhältnissen und/oder durch einen AM Effekt auf das Wachstum gesteigert werden.

Schlagwörter

Allium spec., Ammonium, arbukulärer Mykorrhizapilz, Blütenbildung, Nitrat, ökologischer Gartenbau, organische Schwefelverbindungen, Pelargonie, Poinsettie, Rohphosphat, Salat, Torf-Kompost Substrat

Contents

Chapter 1



1 General Introduction

Horticulture has been practiced since humans became sedentary. Over the years people have learned how to increase the yield of their crops through breeding and fertilization. For the most part, the problems of crop failure regarding nutrient supply have been solved.

Since the 1970s, a new style of plant production known as *organic horticulture* has become increasingly popular. The aim of this new movement was a sustainable ecological system that produces healthy crops. Practitioners of organic horticulture abide by special regulations regarding use of fertilizers and pesticides. The adoption of organic management systems has generated new problems, for example in nutrient supply, that need to be researched.

The organic horticulture movement's goal of producing healthy food for healthy people has contributed to the development of another novel aspect of modern horticulture, the cultivation of plants for their health-promoting phytochemical content. In recent years, it has been discovered that many compounds in crop plants are beneficial to human health. These health related compounds are products of secondary plant metabolism. It was found that the production of these compounds could be influenced by a variety of environmental factors, including plant nutrition and microorganism.

Mycorrhizal fungi belong to a group of microorganisms that are beneficial for plants due to their role in plant nutrient acquisition and stimulation of plant metabolism. In combination with a variety of different fertilizer treatments, it may be possible to use mycorrhizal fungi to solve nutritional problems in organic horticulture and improve the health potential of crop plants. In this endeavor, the form of the fertilizer is an important aspect.

In the following sections, short introductions are given, into the different research areas regarding this work.

1.1 Mycorrhizal symbiosis

The word mycorrhiza was created from the Greek mykes (fungus) and rhiza (root). It describes the mutualistic symbiosis between soil-born fungi and roots of higher plants (Smith and Read, 97). Two main groups of mycorrhizal fungi have been recognized, the ectomycorrhiza and the endomycorrhiza. Endomycorrhizas have been further divided into the arbuscular mycorrhiza, the ericoide mycorrhiza, the arbutoid mycorrhiza, the monotropoid mycorrhiza, the ectendomyccorrhiza, and the orchid mycorrhiza (Peterson et al., 04). Arbuscular mycorrhizal fungi are placed in the *Phylum Glomeromycota;* Class *Glomeromycetes* and are divided into the*,* genera *Acaulospora,*

Archaeospora, Entrophospora, Geosiphon, Gigaspora, Glomus, Diversispora, Pacispora, Paraglomus, and *Scutellospora (*Schüssler, 06; Peterson et al., 04).

The symbiotic association between arbuscular mycorrhizal fungi and plant roots is common in the natural environment. Evidence for the existence of arbuscular mycorrhizal (AM) symbiosis dates back to the Ordovician, 450-500 million years ago (Brundrett, 02; Redecker, 02; Russell and Bulman, 05). Arbuscular mycorrhizal fungi probably already assisted the plants to colonize the land. Today, about 90% of land plants are mycorrhized (Smith and Read, 97) and provide a range of benefits to the host plant, including improved nutrition (Grimoldi et al., 05), enhanced resistance to soil-born pests and disease (Dumas-Gaudot, 00; Whipps, 04), improved resistance to drought (Neumann and George, 04), or salinity (Tian et al., 04), tolerance to heavy metals (Andrade et al., 04; Leung et al., 06; Rivera-Becerril et al., 05), a better soil structure (Piotrowski et al., 04), and earlier flowering (Gaur and Adholeya, 05; Usha et al., 05).

1.1.1 Arbuscular mycorrhizal colonization (mycorrhization)

The mycorrhization of a root starts with the detection by the mycorrhizal spore of signal molecules in the root exudates. These signals, such as flavonoids or strigolactone (Scervino et al., 05; Akiyama et al., 05), dispose the germinated spore to switch from limited to vigorous growth and branching (Tamasloukht et al., 03). As soon as the hyphae contact a root, they form appressioria, penetrate the root surface and colonize the intercellular space of the root cortex. They either enter between two epidermal cells or pass through a cell (Demchenko et al., 04). Recently, Genre et al. (05) found in epidermal cells of *Medicago truncatula,* targeted with *Gigaspora* hyphae the formation of a specific structure composed of microtubules, microfilaments, and cytoskeletal/endoplasmatic reticulum. This structure served as an apoplastic interface compartment through which the fungal infection hyphae traversed the epidermal layer. This penetration of the cortical cells is probably accompanied by the increased production of a cell wall loosening protein (Balestrini et al., 05; Balestrini and Bonfante, 05). Inside the roots the hyphae travel between the cortical cells, but do not enter the central cylinder. In some cells they form tree-like structures called arbuscules by repeated dichotomous branching of the hyphae. The branching of the hyphae results in a two- to four-fold larger surface area, where the main exchange between plant and fungus takes place. The fungi provide nutrients in exchange for carbohydrates. The arbuscules are separated from the cell protoplast by the host plasma membrane, the periarbuscular membrane. The cell undergoes several changes while being mycorrhized. The organelles increase in shape and number, the nucleus moves to the center of the arbuscule,

and the microtubuli and microfilamenti reorganize. After 4-10 days the arbuscule senesces, collapses and its structures are completely degraded (Hause and Fester, 05).

Some fungal species form intra- or intercellular vesicles, which function as storage for lipids (Smith and Read, 97). Hyphae do not only grow within the roots, but also grow out of the root to penetrate the root at a different location or to exploit surrounding nutrients in the soil to contribute nutrients to the plant. These so called extraradical hyphae form spores towards the end of the fungal lifecycle (Hause and Fester, 05).

The observation of morphologically different roots led to a general thesis of Baylis (72) that all plants with thick unbranched roots and few root hairs, like *Allium*, *Coprosma*, or *Citrus,* are apparently more responsive to mycorrhizal colonization than are plants with finely branched roots and long or numerous root hairs when growing in low P soils (Jakobsen et al., 05; Koide, 00). Experiments with hairless mutants revealed that hyphae effectively replace root hairs and therefore support growth of plants with no or poorly developed root hairs (Chen et al., 05).

The host plant is not the only symbiotic partner of mycorrhizal fungi. Bacteria inside or associated with AM imply that many AM symbioses are tripartite associations, and such bacteria support the mycorrhizal effects (Bonfante, 03; Rillig et al., 05; Barea et al., 05; Toljander et al., 06).

1.1.2 Arbuscular mycorrhizal fungi in organic farming

Arbuscular mycorrhiza colonization has often been observed in agriculture soils all over the world, with beneficial effects but also costs for the crop plants (Morgan et al., 05). Many agriculture management systems include use of fertilizers, biocides, tillage, monocultures and growing of non-mycorrhizal crops that are harmful to arbuscular mycorrhizal fungi (Gosling et al., 06). Gryndler (05b; 05a) found that AM growth was inhibited by mineral fertilizer, whereas sole organic fertilizer with humic acids enhanced growth. This finding, together with those of Mäder (02) and Oehl (04; 05) that organically managed sites increase the biodiversity of AM, suggests that fungi could be used advantageously in organic agriculture. Recently, these aspects have been discussed in more detail by Gosling (06).

1.1.3 Characteristics of arbuscular mycorrhizal nutrient uptake

The most frequently reported characteristic of arbuscular mycorrhizal fungi is the phosphorus (P) effect (Lekberg and Koide, 05). It appears mainly on soils that are deficient in P where the plant dry weight was visibly supported by the increased uptake of P (Grimoldi et al., 05; Asghari et al., 05). This increased efficiency of P utilisation compensates the higher plant's construction costs of carbon (C), as soon as the AM fungus is established. Sometimes very young plants can not compensate the C drain and the

plant growth is slowed (Mortimer et al., 05). This can also happen when the soils are not nutrient deficient and plants do not depend on the fungus (Koide and Mosse, 04; Lerat et al., 03). Nutrients that can be transferred to the plant via hyphae beside P are the also relatively immobile zinc (Zn) and copper (Cu), and sometimes also nitrogen (N) and potassium (K) (George, 00; Azcón et al., 03). Magnesium (Mg) and iron (Fe) have also been reported to be transported in higher amounts towards the plant roots (Azcón et al., 03). The main mechanism by which mycorrhizal fungi increase nutrient uptake is through more extensively soil utilization rather than a unique capacity to mobilize nutrients that are not available to plants (Sanders and Tinker, 71, Haymann and Mosse, 72; Drew et al., 03). AM hyphae have been shown to link plant roots and also to translocate P between the same or different plant species (Yao et al., 03).

1.1.3.1 Uptake of phosphorus

Phosphorus in soil can be categorized as either inorganic or organic. Inorganic P is often integrated in crystal lattices with Ca, Fe and Al that form largely insoluble complexes, or absorbed to the surface of clay minerals. Plant available P consists of loosely bound P that exchanges relatively rapidly with the soil solution at pH 6.5. Tightly bound P exchanges very slowly with soil solution and is regarded as unavailable to plants. Organic P derives from soil organisms, including plants, microorganisms and animals and can be extracted from soil mainly as inositol phosphates (PO_4^{3-}), phospholipids and nucleic acids (Smith and Read, 1997). Plants, and potentially AM as well, can secrete phosphatases to help hydrolyze this organic PO_4^{3-} (Joner and Johansen, 00; Koide and Kabir, 00). The uptake of PO_4^{3-} by plants is regulated by the concentration and the electrical gradient between internal and external conditions. Two transporter systems exist; one is a low affinity transporter system working constantly and the other is a high affinity uptake system that is strongly enhanced through P deficiency (Raghothama and Karthikeyan, 05).

The active uptake of P by AM and its transport as short chain poly P via hyphae to plant roots is influenced by the transfer of carbon, as hexose, from the host to the AM across the mycorrhizal interface. Within the extraradical hypae P is stored in fungal vacuoles in form of short- and long-chained poly P (Bücking and Shachar-Hill, 05).

In *G. intraradices* for example, the uptake of P is regulated by the P transporter gene GiPT in the extraradical hyphae. The expression of this gene in turn is regulated by external P concentration, but probably also by the internal P status of the plant (Maldonado-Mendoza et al., 01), and by the N supply (Olsson et al., 05a). The transfer of P from fungi to the plant has been studied at the molecular level in several plant species (Rausch et al., 01; Nagy et al., 05; Poulsen et al., 05).

In conclusion, the advantages of mycorrhizal hyphae in P acquisition are that they (1) can transport P much faster to the plant than by diffusion in soil, (2) overcome the depletion zone around roots, (3) use less C for their construction than for the same root length, (4) can explore a larger soil volume by penetrating pores of smaller diameter than roots (Drew et al., 03), and (5) are more competitive against free-living soil microorganisms for recently mineralized or solubilized Pi than are roots (Smith and Read, 97).

1.1.3.1.1 Uptake of P from rock phosphate

In organic agriculture and horticulture the application of P fertilizer is allowed only in the form of organic materials (e.g. chicken manure) or as rock phosphate. Rock phosphate is a raw rock mined from P-rich deposits. The rock is washed free from clay impurities, heated to remove moisture, and ground (Espoma, 06). Rock phosphate is a slowly soluble fertilizer (Steffens et al., 06; El Dessougi et al., 03) and has a much lower degree of efficiency than chemically decomposed P fertilizer (Steffens et al., 06). Mycorrhiza fungi have been reported to increase P uptake from rock phosphate in roothairless barley roots (Chen et al., 05) and to increase the yield of *Alfalfa* significantly (Barea et al., 02). On acidic soils fertilized with rock phosphate, arbuscular mycorrhiza increased shoot and root dry weight and P, Cu, Zn, B, Mg, Ca, and K concentrations of *Zea mays* L. (Alloush and Clark, 01). A combination of mycorrhizal fungi and P solubilizing bacteria or organic matter were found to be very efficient in exploiting the rock phosphate (Barea et al., 75; 02). Probably the hyphae immediately take up inorganic P that is dissolved in the soil solution during mineralization of organic P by microorganisms, and prevent its sorption on clay minerals (Jakobsen et al., 94). Lowered soil pH, which may result either from CO_2 entry into the soil or from use of ammonium fertilizer, can also contribute to the availability of P in the soil solution (Li et al., 91; Son et al., 06).

1.1.3.2 Uptake of N, especially ammonium and nitrate

The development of AM hyphae is dependent on a sufficient N supply (Hawkins and George, 01). Extraradical hyphae of AM take up ammonium (NH_4^+), nitrate (NO_3^-), and amino acid via transporters/permeases and proton pumping ATPases (Breuninger et al., 04). Recent experiments have shown that NO_3^- and NH_4^+ are assimilated into arginine at the tip of the hyphae, transported to the plant, and transferred probably as NH_3 at the fungus-plant interface (Govindarajulu et al., 05).

It has been shown for ammonium that NH_4^+ nutrition can lower the rhizosphere pH and suppress spore germination (Green et al., 76), root colonization, and growth of AM (Ortas and Rowell, 04), and can also directly reduce root and/or extraradical hyphal biomass (Hawkins and George, 01; Olsson et al., 05a). Olssen et al. (05a) found that N

availability can regulate nutritional processes in AM. The suppressing influence of a high nutrient supply on AM could be a result of increased C immobilization in the plant. In this case the N available to the plant may reduce C-flow to the AM in a similar way as P availability does (Olsson et al., 05a).

1.1.4 Changes of secondary metabolism by AM: phytohormones and flowering

It has been shown that AM can enhance phytohormones (Barker and Tagu, 00) like abscisic acid (Danneberg et al., 92), the auxin conjugate indole-3-butritic acid (Ludwig-Müller et al., 97; Fitze et al., 05), cytokinin isopentenyl adenosine (Shaul-Keinan et al., 02), hydroproline-rich glycoproteins (Van Rhijn et al., 97), jasmonic acid (Hause et al., 02), and gibberellin-like compounds (Allen et al., 82; Danneberg et al., 92; Shaul-Keinan et al., 02). Most of these observations were made in mycorrhized roots, but some phytohormones like cytokinin or auxin conjugates have also been monitored in the shoot (Shaul-Keinan et al., 02; Fitze et al., 05).

Flower development is a process highly influenced by phytohormones. AM has been shown to increase flower development and lead to earlier flowering (Backhaus, 83; Gaur and Adholeya, 05; Usha et al., 05). Although this phenomenon has often been observed, there has not been much work done to understand the mechanism. From molecular studies with *Arabidopsis thaliana* it has been shown that flower development is influenced by the environment and by phytohormones like auxin and gibberellin (cp. 2.3.4.1). Even though this test plant is not colonisable by AM, observed increases in gibberellin and auxin concentrations in mycorrhizal plants could be responsible for their earlier flowering.

1.1.4.1 Mechanisms of flower development

The most detailed outline of flower development has been given in the model plant *Arabidopsis thaliana*. Many mutants have been developed from this species that made it possible to explain the molecular mechanisms underlying flower development and give an overview picture (Krizek and Fletcher, 05; Jack, 04). The general model used is the ABC model, in which the LEAFY (LFY) gene has the central role. Flower development can be divided into four steps: First, the plant switches from vegetative to reproductive growth after receiving certain environmental and developmental signals, such as long-day photoperiod, gibberellins (Yu et al., 04) (important for short day), autonomous promotion, and vernalization (cold treatment). Second, a small group of meristem identity genes that specify floral identity are activated by signals from several flowering pathways. Third, these meristem identity genes activate the floral organ identity genes in a specific region of the flower. Fourth, the floral organ identity genes activate

genes that induce different cell types to "build" the floral organs (Jack, 04) (Fig. 1). The phytohormone auxin takes part in the selection of the sites of the primordium specification (Bénkova et al., 03; Reinhardt et al., 03; Reddy et al., 04).

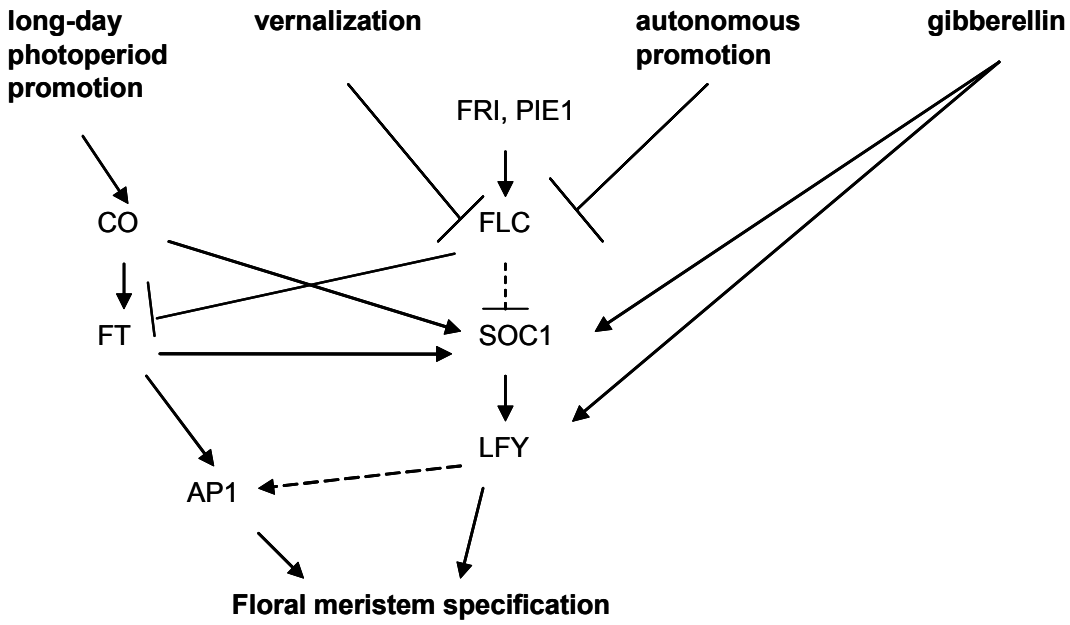


Fig. 1: The major floral inductive pathways in Arabidopsis. Signals from four major inductive pathways are integrated by FLC, SOC1, FT and LFY. Interactions are shown as ┤ for a repressive signal or → for the positive regulator signal. Abbreviation: CO: Constans gene; FT: Flowering locus T; AP1 and LFY: major floral meristem identity genes; FRI: Frigida gene; PIE1 photoperiod independent earlier flowering 1; FLC: Flowering locus C, low FLC early flowering, overexpressed FLC late flowering; SOC1: suppressor of overexpression of CO (after Jack, 04; Moon et al., 05).

The major floral meristem identity genes in *Arabidopsis thaliana* are LFY and Apetala 1 (AP1). The activation of these two genes results, either directly or indirectly, from the outputs of the flowering time pathways. The signal of gibberellin is directly integrated by LFY, whereas the others are integrated upstream or in parallel to LFY by flowering locus C (FLC), suppression of overexpression of constans (SOC1), and flowering locus T (FT). Long-day-photoperiod and gibberellin induce SOC1 (Moon et al., 03).The floral repressor FLC integrates the repressive signals from the autonomous and vernalization pathway, and the positive regulatory signals from the gene Frigida (FRI) and PIE1. High levels of FLC correlate with late flowering, and low levels of FLC correlate with early flowering. One signal on the autonomous pathway, for example, is a protein with similarity to a component of the histone deacetylase complex of mammals (He et al., 03). The up regulation of SOC1 via FLC can only take place when either gibberellin or long-day photoperiod is also present. The second pathway that long-day photoperiod activates is over FT that rather activates AP1 than LFY. LFY activity is necessary for the proper expression of floral organ identity genes, although it does not function

independently of AP1. AP1 is probably directly activated by LFY (Jack, 04; Wagner et al., 04).

1.2 Plant mechanisms for uptake and assimilation of nitrogen und sulfur

Membrane transport of anions and cations is carried out via ion selective transporters or through channels. Potassium is taken up actively but most other cations pass passively through the cell membrane along the electronic potential. Anions are moved out with the efflux pump to keep down sodium (Na) and calcium (Ca) concentration in the cell. External and internal signals attach to the receptors of the membrane transporters, which convert and start the transport process (Marschner, 95). The activity of root NO_3^-, NH_4^+ and sulfate (SO_4^{2-}) transporters are likely to be linked to changes in sucrose transfer to the roots (Lejay et al., 03).

1.2.1 Sulfate uptake

Sulfate is a macronutrient for plants that is essential for the synthesis of sulfur-containing amino acids (Leustek and Saito, 99). Molecular studies have shown that most of the higher plants have high-affinity SO_4^{2-} transporters that potentially assist the uptake of SO_4^{2-} (Smith et al., 95; 97; Takahashi et al., 97; Hawkesford, 03). In addition to sulfate transporters used for uptake, there are voltage-dependent channels in some tissues that are initiated by SO_4^{2-} and inactivated by nucleotides (Frachisse et al., 99). These plasma membrane-bound SO_4^{2-} transporters are located in the surface cell-layer of roots and function as an energy dependent proton/sulfate co-transport system that can be initiated especially at times of SO_4^{2-} limitation in the roots. Once within the plant, SO_4^{2-} is reduced to sulfide (S_2^-) (1) (Lüttge et al., 99). For excess SO_4^{2-} the vacuole is the major intracellular storage (Hawkesford and Wray, 00).

(1) $SO_4^{2-} + 8e^- + 8H^+ \rightarrow S_2^- + 4H_2O$

1.2.2 Nitrogen uptake

In the soil, nitrogen (N) is extremely heterogeneously distributed and consists of a complex mixture of organic and inorganic forms (Miller and Cramer, 05). The N content required for optimal growth depends on the plant species, development stage, and organ. It varies between 2 and 5% of the plants dry weight (Marschner, 95; Miller and Cramer, 05). Nitrogen is present in proteins, nucleic acids, coenzymes and numerous secondary plant compounds. The size and architecture of the root system is of relevance for a sufficient access to N sources (Miller and Cramer, 05). Generally, N is assimilated

by NH_4^+, NO_3^-, or amino acid uptake, or by N_2^- fixation. Of these speciations, inorganic N is probably the dominant N source for crop plants. The reason for this is that organic N in the form of amino acids has a low diffusion coefficient and is rapidly turned over by micro organisms, and hence is taken up by the roots at a very low rate (Owen and Jones, 01). The uptake of inorganic (NO_3^- and NH_4^+) and organic N from the soil into roots is realised by multiple transport systems that are mainly situated just behind the root tip. The transporters are induced by several factors, and are subject to complex regulation at the level of transcription, translation and post-translation (Miller and Cramer, 05).

1.2.2.1 Nitrate uptake and assimilation

Nitrate is preferentially taken up by crop plants, because NO_3^- is generally provided in higher concentrations than either NO_2^- or NH_4^+ (Miller and Cramer, 05). Nitrate is mainly delivered to the roots through a combination of mass flow and diffusion, and depends on soil moisture, soil impedance and root uptake and growth rate (Craine et al., 05). Plants take up NO_3^- in cotransport with H^+ (Ullrich and Novacky, 90). Uptake is regulated by high- and low affinity NO_3^- -uptake systems that operate at different external NO_3^- concentrations (Aslam et al., 92; Glass and Siddiqi, 95). At low external NO_3^- concentration (<0.5 mM), two high-affinity transport systems probably assume most of the responsibility for N uptake, whereas at high NO_3^- concentration (>0.5 mM), one low-affinity transport system does the work (Glass and Siddiqi, 95). Besides physiology studies, many investigations at the molecular level have been conducted recently on uptake and transport mechanisms (e.g. Tong et al., 05; Berger et al., 06; Little et al., 05).

At low NO_3^- supply, most nitrate is either reduced by NO_3^- and NO_2^- reductase to NH_4^+ within the root cells ((2) and (3)) (Lüttge et al., 99) or stored in the vacuoles (Miller and Cramer, 05; Granstedt and Huffaker, 82).

(2) $NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O$ (in cytoplasma)

(3) $NO_2^- + 6e^- + 6H^+ \rightarrow NH_3 + H_2O + OH^-$ (in chloroplast)

Increasing NO_3^- concentration in the soil solution results in a partial transport of nitrate into the shoots (Britto and Kronzucker, 05; Marschner, 95; Andrews, 86). It enters the xylem by anion channels or via the voltage dependent Quickly Activating Anion Conductance (X-QUAC) and is transported with endogenous K as a counterion (Köhler et al., 02; Marschner, 95).

1.2.2.2 Ammonium uptake and assimilation

Compared to NO_3^-, NH_4^+ is relatively immobile in the soil (Miller and Cramer, 05). Ammonium uptake may occure through channels, even through K channels, driven by negative membrane potential of the plant cell (White, 96). Entery of NH_4^+ into cells may be in countertransport with protons (Britto and Kronzucker, 05). For closer investigations many plant NH_4^+ transporter (*AMT*) genes have been identified that could take part in the uptake of NH_4^+ (Miller and Cramer, 05). Compared to NO_3^- assimilation, NH_4^+ assimilation increases carbon (C) utilization for respiration and amino acid production (Britto and Kronzucker, 02b).

Once within the cell, most NH_4^+ is dissolved quickly into ammonia ($NH_3 + H^+$), integrated directly into amino acids and amides (Schilling, 00), and transported in the xylem to the shoot mainly as sucrose. This synthesis of amino acid (glutamine and glutamate) requires a large amount of C skeletons from α-ketoglutarate, ATP and reducing power (Ferrario-Méry et al., 05). Translocation of low NH_4^+ concentrations in the xylem has been measured (Schjoerring et al., 02), but the loading mechanism has not been clarified yet (Miller and Cramer, 05).There is also evidence that NH_4^+ may be stored in vacuoles of the roots were it raises the pH with increasing external NH_4^+ concentration (Roberts and Pang, 92).

High concentrations of NH_4^+ can be toxic to some species. Several reasons for this have been postulated, including internal decrease of the pH (pH imbalance) (Van den Berg et al., 05), external acidification (Britto and Kronzucker, 02b), cation deficiency (Van Beusichem et al., 88; Lucassen et al., 03), and energy drain resulting from the efflux/pumping process of NH_4^+ (Britto and Kronzucker, 02b). Plants react to NH_4^+ toxicity by increasing the efflux/influx ratio of NH_4^+ in leaves and root cells (Britto et al., 02a).

1.2.2.3 Competition between ammonium and nitrate uptake

In almost all cases external NH_4^+ strongly suppresses net uptake of NO_3^- (Kronzucker et al., 99a). In contrast, externally supplied NO_3^- generally has little or no effect on net uptake of NH_4^+ (Marschner, 95), but activates NH_4^+ transporters genes at NO_3^- deficiency (Wang et al., 00). Accordingly, by supplying NH_4NO_3, NH_4^+ is usually taken up much more preferentially than NO_3^-, accompanied by an optimal growth of most plants species at mixed supply of NH_4^+ and NO_3^- (Marschner, 95). One explanation for the suppression of net NO_3^- influx by NH_4^+ in the plasma membrane could be the inhibition of the inducible high-affinity transport system and on a very small scale also NO_3^- efflux (Kronzucker et al., 99a). A second explanation could be the energy balance. The processes of NO_3^- reduction and assimilation have a higher energy requirement when carried out in roots than that of NH_4^+. Expressed in ATP equivalents, 15 moles of ATP are

used for the reduction of one mole of NO_3^-, with an additional 5 moles of ATP for ammonia assimilation (Marschner, 95). The assimilation of NO_3^- in barley roots hence requires 9% more energy than when NH_4^+ is supplied, but NH_4^+ in high concentration is toxic (Bloom et al., 92). The reason for the optimal growth of most plants at the mixed supply of NH_4^+ and NO_3^- could be that they complement one another. This effect may result from balancing the pH process in the cytosol and the external soil solution (Britto and Kronzucker, 05), translocating more N into the shoot (Kronzucker et al., 99b), and possibly inducing assimilating pathways in the roots by NO_3^- that are normally not available with NH_4^+ (Britto et al., 02b).

1.2.3 Sulfur and nitrogen interactions

Sulfur transporters are basically regulated by external S conditions, but are additionally controlled by plant C status and N supply (Fig. 2) (Maruyama-Nakashita et al., 04). These act as signals which activate molecular mechanisms that modify biosynthetic pathways and thereby have a profound impact on metabolite fluxes (Hesse et al., 04). In *Arabidopsis* roots, C supply generally induces the expression of nutrient transporters, especially nitrogen (Lejay et al., 03; Palenchar et al., 04). Apparently, the nutrient uptake systems are co-ordinately operated under a general regulatory circuitry to meet the demands of primary metabolism when C is sufficiently supplied. The exact mechanism remains an open question (Maruyama-Nakashita et al., 04).

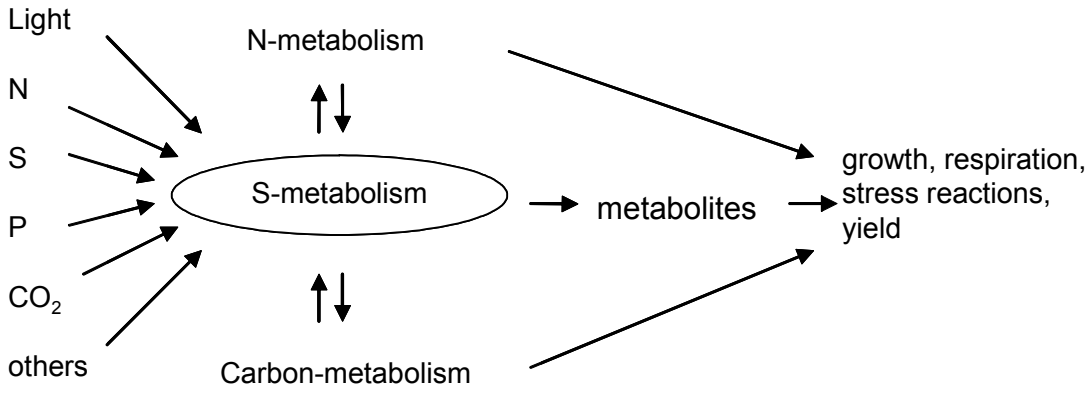


Fig. 2: Plant metabolism is highly interconnected and dependent on external supply of nutrients, light and water. Scheme showing factors affecting sulfur-assimilation and the interrelation with nitrogen and carbon metabolism in plants. The regulatory and biosynthetic circuits lead to the respective composition of plant metabolites and, eventually, to plant growth and reproduction (Hesse et al., 04).

Sulfur uptake and assimilation is dependent on the constant supply of the precursor of cysteine, O-acetylserine (OAS), which, in turn, is controlled by N and C availability (Koprivova et al., 00, Kopriva et al., 02). Excess cysteine and glutathione (GSH) con-

centrations induced the down-regulation of the SO_4^{2-} transporter, with an accompanying rapid decrease in SO_4^{2-} uptake. This can happen when either S is in excess, or N is limited (Smith et al., 97; Zhao et al., 99).

Cysteine is the first molecule in the plant metabolism containing both S and N. Cysteine is incorporated into proteins and glutathione and functions as the main S donor for methionine synthesis (Ravanel et al., 98; Hesse and Hoefgen, 03; Leustek and Saito, 99). Cysteine acts as a general catalyst in redox reactions. Furthermore, secondary compounds such as S-alk(en)ylcysteine, S-methylcysteine, glucosinolates, and phytoalexins are based on S directly or on cysteine and methionine, respectively (Schmidt and Jäger, 92). S-alk(en)yl cysteine sulphoxides are the precursors to health related organosulfur compounds of *Allium* species (Jones et al., 04). Limitations in either nutrient do not only inhibit the plant's ability to synthesize cysteine, but also limits protein synthesis. Clarkson (89) observed a suppressed uptake of NO_3^- and NH_4^+ when cereal plants had S deficiency.

Cysteine is formed in a stepwise process starting with SO_4^{2-} uptake by the appropriate SO_4^{2-} transporter (Fig. 3), followed by the activation of SO_4^{2-} by covalent binding to ATP via an ATP-sulfurylase-catalysed reaction to form APS, its reduction to sulfite by APS-reductase (APR), and finally the reduction to sulfide by sulfite reductase. The O-acetylserine(thiol)lyase (OASTL) transforms sulfide and activated serine, O-acetylserine (OAS), into cysteine. The OAS is synthesized by serine acetyltransferase (SAT) which forms a complex with OASTL. These two enzymes catalyse the final step of the cysteine biosynthesis and represent the major link between N/C and SO_4^{2-} assimilation. This multi-enzyme complex is called cysteine synthase (Hawkesford and Wray, 00, Leustek and Saito, 99). The regulation of SATs was shown in different plant species, including *Allium tuberosum* (Urano et al., 00).

The effect of S deficiency on N metabolism is much less obvious. Hesse (04) speculates that the interrelationship of S and N metabolism is of a hierarchical nature, where N metabolism has priority over S metabolism.

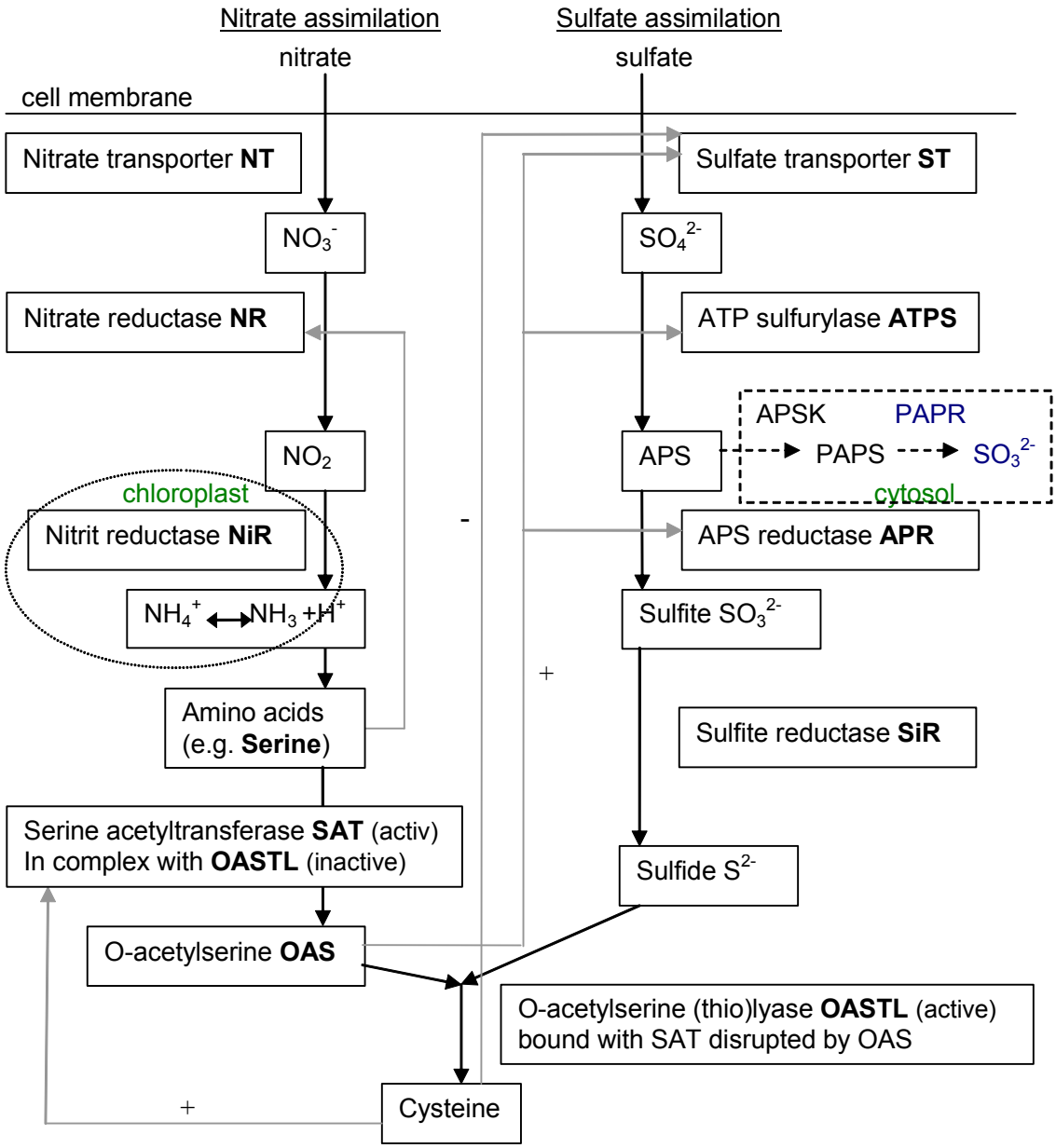


Fig. 3: External sulfate is taken up through sulfate transporters. The inert sulfate is activated by covalent binding to ATP to form APS either in the cytosol or the plastid. In the cytosol APS can be phosphorylated to PAPS, in chloroplasts, sulphate bound in APS is reduced to sulfide via sulfite and subsequently transferred to activated serine (OAS) to form cysteine. Cysteine formation takes place in three cellular compartments, chloroplasts, but also cytosol and mitochondria. In these compartments both SAT and OASTL isoforms are present but the reductive component of the pathway is missing. Black lines represent metabolic efflux; grey lines are probable feedback control loops. ATP adenosinetriphosphat; ADP adenosine-5'-phosphosulphate; APSK APS kinase; PAPR PAP-reductase; PAPS phosphoadenosine-phosphosulphate (Hesse et al., 04; Lüttge et al., 99).

1.3 Formation of organosulfur compounds and their contribution to human health

The health related organosulfur compounds of *Allium* species can be easily recognized by their flavour. The nature and origin of these flavour compounds have been

studied since the 1940s. It was shown that once the plant tissue is damaged, stable flavour precursors react to form a series of volatile and non-volatile S compounds. These compounds belong to the group of secondary metabolites which function in key activities including reproduction, defence, pathogenicity, stress resistance and resource storage (Jones et al., 04).

The cysteine and glutathione metabolisms are involved in biosynthesis of organosulfur precursors in the *Alliums*. Therefore, I focus on the biosynthetic pathway of the health related flavour precursors, the (+)-S-alk(en)yl cysteine sulphoxides (CSOs) and their γ-glutamyl peptide (γGPs) relatives decribed by Jones (04).

There are four relatively stable, odourless COSs precursors in the different *Allium* species (Fig. 5) known as S-methyl cysteine sulphoxide (MCSO, methiin; present in most Alliums, some Brassicaceae), S-allyl cysteine sulphoxide (ACSO, alliin; characteristic of garlic), S-transprop-1-enyl cysteine sulphoxide (PeCSO, isoalliin; characteristic of onion), and S-propyl cysteine sulphoxide (PCSO, propiin; in onion and related species). After cleavage, the vacuolar enzyme alliinase (EC4.4.1.4.) rapidly lyses them to form sulfenic acids (R-SOH), which immediately condense to form pyruvate, ammonia and the alkyl alkanethiosulfinates (R-SS(O)-R) (Fig. 4; Lawson, 93; Jones et al., 04).

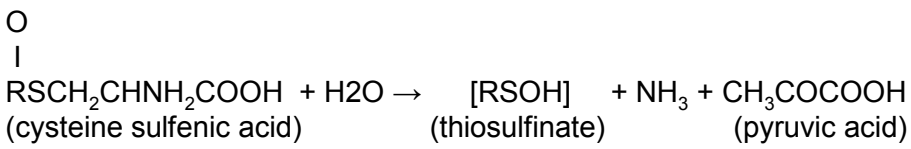


Fig. 4: Reaction from cysteine to sulfenic acid and pyruvic acid (Schwimmer and Weston, 61).

The thiosulfinates are very unstable and continue to react, which is traceable by the changing smell (Lawson, 93). Allicin is one of the compounds that undergoes chemical reaction and quickly transforms into other organosulfur compounds including alk(en)yl, ajoenes and vinyldithiins (Kodera et al., 03). The lachrymatory factor is following the alliinase action on PeCSO and is typical for onions (Imai et al., 02). PeCSO was found to correlate with pyruvic acid concentration in onions, while pyruvic acid concentrations was correlated with S concentrations in the nutrient solution (Randle et al., 95).

Beside CSOs, a number of γ-glutamyl peptides (γGP) derivates of the described flavour compounds have been identified within *Allium* species (Whitaker, 76). They do not contribute directly to flavour, but are part of the biosynthesis and may function as reservoirs for N and S.

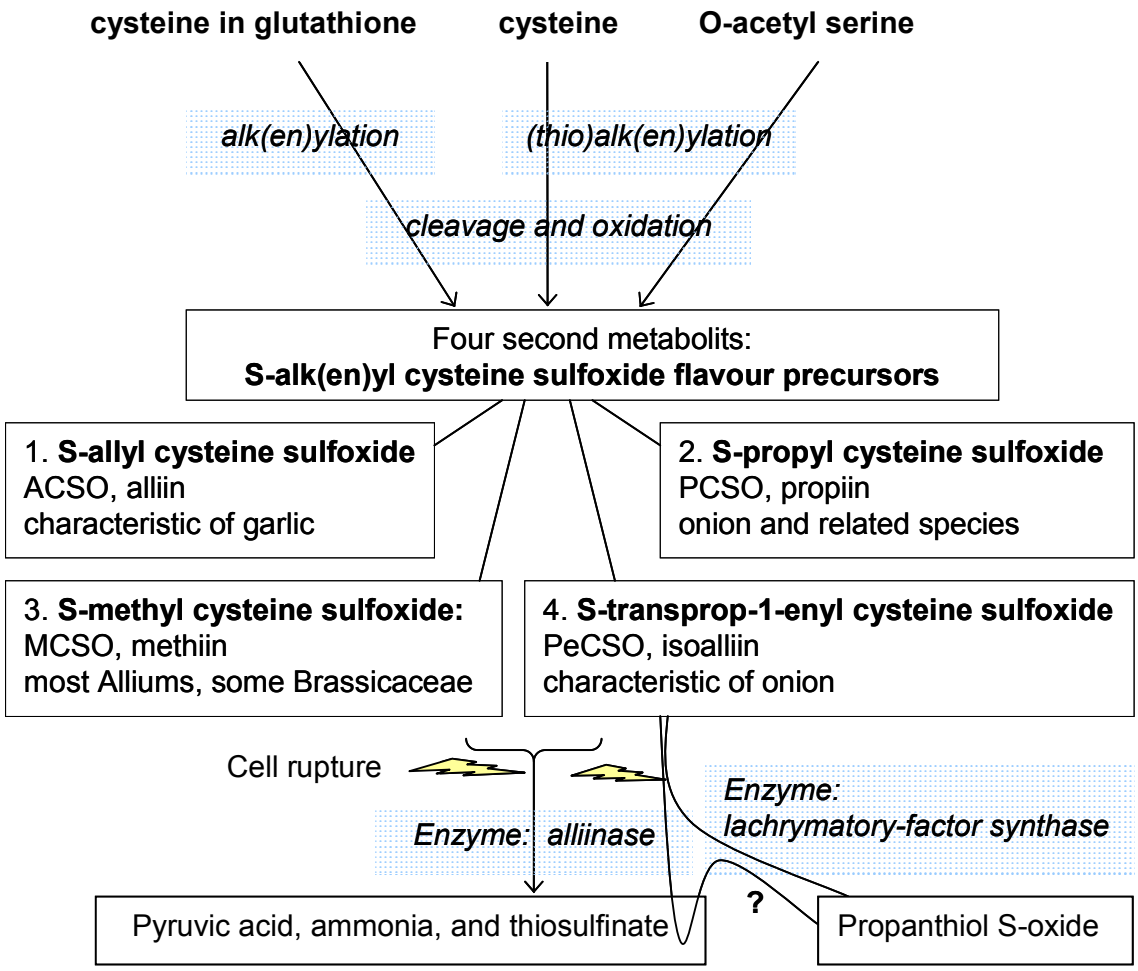


Fig. 5: Biosynthetic pathway of cysteine to thiosulfinates

For human consumption, the organosulfur compounds are of particular interest, because they have several good attributes assigned. They are suggested to be biologically active as antibiotics, as agents in reducing the risk factors of cardiovascular disease, and as blood lipid-reducing agents. They also are gaining growing interest as potential anticancer agents (Lawson, 93; Valli and Giardina, 02; Kodera et al., 03). These health related compounds have been mainly investigated in garlic and onion. For Allium species, the S-allyl group might be the key structure responsible for the biological activity (Goldman et al., 96; Kodera et al., 03). Recently, the biological activity of water-soluble organosulfur compounds extracted from garlic, such as S-allyl-L-cysteine and S-allylmercapto-L-cysteine, have become of public interest because they are stable, odourless and safe (Kodera et al., 03).

1.4 Characteristics of the test plant species

1.4.1 Alliaceae

The family *Alliaceae* contains about 700 species, including economically important vegetables and flowering ornamentals as well as wild species from Europe, Asia, and America (Fenwick and Hanley, 85). They originated in the mountainous region of central Asia. They are perennial plants that can develop roots, rhizomes and especially bulbs as an organ of storage and develop succulent adventitious roots without hairy roots. They have a characteristic *Allium* odour and flavour that is aroused by steroidic saponines (S containing antibacterial oils, e.g., Alliin, Allicin) and chelidonic acid. Most *Allium* species preferentially grow in open, sunny, aridic habitats of the warm temperate zone, but many have adapted to other climates. They can be found at high latitude (chive up to 70° n. latitude) and in tropical areas. Some of them have summer (onion, garlic) and some have winter dormancy (chive). *Allium* plants are often colonized by arbuscular mycorrhizae.

1.4.1.1 Chinese chive (Allium tuberosum Rottler ex Sprengel)

Chinese chive has been used for culinary and medicinal purposes. Three horticultural products are harvested: the green and blanched leaves, and the closed flower buds. All parts of the plant have a mild garlic flavor and are mainly used as a fresh culinary herb (Larkom, 94). The leaf length (20 cm), dark green colour, flavour, and tenderness are quality parameters. The rhizome essentially replaces the poorly formed bulb as the storage structure (Rubatzky and Yamaguchi, 97).

Chinese chive is propagated from seeds and grown in beds as a semi-permanent crop for 4 to 5 years (Cantwell et al., 96). The leaves grow best at 20 °C (Rubatzky and Yamaguchi, 97).

1.4.1.2 Bunching onion

Chinese bunching onion (*Allium fistulosum* L.) is used in the kitchen for its flavor and mild pungency. The seeds are sown from spring until summer and the plants are harvested in the following winter until spring or summer. If wanted they are earthed up during growth to get a long white stalk. Bunching onion is hardy and can be harvested throughout the year. It is harvested at several stages, depending if the green young leaves or the white stem are preferred. The pseudostems vary in length and thickness, but can be up to 50 cm long and 3.5 cm wide (Brewster, 94; Kitazawa, 06).

1.4.1.3 Leek

Leek is not known as a wild plant and was probably cultivated from *A. ampeloprasum* L. for culinary purposes. Seedlings are planted on a bed system from early spring until late July. They are earthed up during growth to reach a higher length of blanched sheath. Similar to bunching onions, the leek plant is a very hardy crop and in many parts of Europe it can stand in the field and be harvested throughout the year. Optimal growth conditions are reached at temperatures about 22 °C combined with a good water supply (Brewster, 94).

1.4.2 Lettuce

Lettuce is an annual vegetable (*Lactuca sativa* and varieties) of the family *Asteraceae*. It probably originated in the East Indies or Asia Minor and possibly descended from wild lettuce (*L. scariola*). *L. sativa* has been grown as a salad plant since antiquity. Three different kinds of lettuce are cultivated: head, or cabbage, lettuce; the leaf, or loose, type; and Cos lettuce, or romaine (Encyclopedia, 03). Lettuce (*Lactuca sativa*) seeds are germinated at 10 °C and transplanted from early spring until summer and harvested starting from May. Cabbage lettuce is slower in growth, whereas the outer leaves of loose-leaf lettuce can be harvested earlier and repeatedly. Optimal growth conditions are reached at temperatures about 15-20 °C (Baake, 06).

1.4.3 Pelargonium

Wild pelargonium (*Pelargonium peltatum*) plants originated in the South African region, where they grow in the full sun and are exposed to periodic dry seasons. Horticulturists have bred about 250 species that are mainly cultivated as hybrids. Pelargonium plants are mainly propagated by rooting tip cuttings from branches of vegetative stock plants at air temperatures of 16 °C to 19 °C in a greenhouse. After 16-20 days, they are transplanted and the temperature is decreased to 15 °C. Six weeks later they have their first buds and can be sold fully in flower after 8 weeks. The substrate usually used is a mixture of compost, peat, and clay (Elsner et al., 95; Zimmer, 91).

1.4.4 Poinsettia

Wild poinsettia (*Euphorbia pulcherrima*) plants originated in the plateau of Mexico with a mean annual temperature of 18 °C and thrive and prosper on shady and moist sites. Poinsettia plants are long-night plants whose flowering is initiated when day length is reduced to a critical point (12 h and 20 min). If the day length is reduced continuously, colourful bracts surround the flowers. They are sold normally around Christmas time. Optimum day temperatures are usually 21 °C to 29 °C and in the night 16 °C to 21 °C. Poinsettia plants are propagated by rooting tip cuttings from branches of

vegetative stock plants around July and August. To generate a vigorous root system they require 3-4 weeks. The substrate can be a mixture of sand, peat, perlite, vermiculite, or bark (Ecke et al., 90; Zimmer, 91).

1.5 Compost

Compost consists mainly of partly decayed organic material. It is used as a fertilizer or as a soil ameliorant for increasing humus content in vegetable farming, home gardens, flowerbeds, lawns, and greenhouses. Compost is usually composed of plant materials (e.g., grass clippings, vegetable tops, garden weeds, hay, tree leaves, sawdust, and peat) mixed with manure and soil (Encyclopedia, 03). Compost quality can be rated based on a variety of compost properties. High quality compost has several requirements that have to be achieved. Quality criteria for composts are specified in Germany in various regulations (Stöppler-Zimmer et al., 93; Anonymus, 98). These include the following aspects: clearance certificate of epidemic hygiene, maximum allowed impurities, portion of stones, plant compatibility, degree of decomposition, water content, organic matter, parameters subject to declaration such as class and structure of compost, maximum grain size, raw density, salt content, pH-value, plant nutrients, heavy metals etc. (Hams and Becker, 99). For plant cultivation in horticulture, compost can have a positive effect on plant yield and quality due to supply of humus, fertilization attributes, improvement of soil-physical properties and the cation exchange capacity, increase of the soil pH, and biological soil activity (Stöppler-Zimmer et al., 93). The different characteristics can be influenced by the ingredients of the compost. The salt concentration for example, can be reduced by amendment with green residues like wood and branches, but grass residue increases it. The KCl concentration is normally between 3 and 8 $g \cdot L^{-1}$, while that of NaCl is usually lower. Typical values for available macronutrient concentrations ($mg \cdot L^{-1}$) of composts from green residues are: N, 50 to 200; P, 109 to 305; K, 415 to 1245; and Mg, 150 to 300; with pH between 6.5 and 8.5 (Stöppler-Zimmer et al., 93).

1.6 Research focus

This thesis focuses on the interface of science and praxis. It investigates the prospects of arbuscular mycorrhizal fungi, in combination with various fertilizers to contribute to growth, nutrient uptake, flowering, and production of secondary metabolites of horticultural plants. One main emphasis of the present work was to address issues of current importance to organic horticulture.

Four main topics are included in the work:

a) Mycorrhization

Vegetables and ornamental plants are often cultivated on peat-based substrates or in semi-hydroponic systems with, e.g., perlite. In organic farming the use of peat substitutes, like compost, is encouraged. As examples of different cultivation systems, peat-compost substrates and perlite were used. All of the chosen substrates were expected not to contain any mycorrhizal infectious material. It was therefore of interest if a mycorrhization of different plant species was possible on these substrates.

b) Plant growth and nutrient uptake

Plant growth and nutrient uptake depend on the mycorrhizal species and on the nutrient availability of fertilizers. Several different AM inocula can be purchased commercially. Besides three different commercial inocula, two single strains isolated from organically managed and natural habitats were tested for their contribution to growth and nutrient uptake of the plants. Both, plants and AM, also rely on the fertilizer form that is supplied. Therefore, several fertilizers were tested, including organic fertilizers like compost and horn meal, and the mineral fertilizers rock phosphate, sulfate, ammonium, and nitrate.

c) Flowering

Flower development is important for ornamental crop production. Earlier flower development of mycorrhized plants has often been observed by scientists. This was explained by a higher nutrient supply or hormonal induction. An experiment was conducted with two ornamental plants under organic management conditions with compost and horn meal as fertilizers.

d) Plant secondary metabolites

Mycorrhizal fungi have been reported to influence secondary plant metabolism. As *Allium* species are known for their health related compounds and dependency on mycorrhizal fungi, they were chosen to investigate the contribution of mycorrhizal fungi to plant composition. The flavor and health related compounds of *Allium* plants consist mainly of organosulfur compounds. These organosulfur compounds can be influenced by sulfur and nitrogen fertilization. Broadly speaking, the more sulfur, the more organosulfur compounds. Recent experimental designs though have not taken the unspecific competition between sulfate and nitrate uptake into account. Therefore different ammonium and nitrate ratios were chosen for the experiments. Moreover, a high concentration of ammonium in the external solution has been reported to be toxic for plants. In contrast, ammonium is the most important source of mineral nitrogen for

microorganisms. Therefore arbuscular mycorrhizal fungi were included in the experiment to test their influence not only on the secondary plant metabolism but also on plant growth.

From these main topics the flowing hypotheses were generated:

AM will colonize plant roots on peat, peat-compost and perlite substrates.

AM will increase shoot dry weight and shoot nutrient concentrations on peat substrates with low P available fertilizers, with higher compost amendments, and on perlite with a high ammonium:nitrate ratio.

- AM colonization will increase P mineralization from rock phosphate on peat substrates.
- Single AM strains originating from organically managed soils will be superior in the mobilization of P from rock phosphate compared to AM strains from natural habitats or an all purpose horticulture commercial mixed inoculum.

AM will increase flower and bud development.

In *Allium* plants fertilization with a higher ammonium:nitrate ratio, AM will increase plant growth, stimulate the secondary plant metabolism, and consequently increase plant production of organosulfur compounds.

An increased ammonium:nitrate ratios in the external solution will increase the uptake of sulfate and therefore increase the production of organosulfur compounds in Allium plants.

This work was done in cooperation with the Research Institute of Organic Agriculture (FiBL) in Switzerland, the Faculty of Organic Agricultural Sciences at the University of Kassel in Germany, and the Chinese Agricultural University of Bejing in China.

Chapter 2

2 Effect of Mycorrhizal Inoculation and Compost Supply on Growth and Nutrient Uptake of Young Leek Plants Grown on Peat-based Substrates

2.1 Abstract

Organic horticultural production systems often are characterized by the use of beneficial soil micro-organisms because the application of soluble inorganic P or N fertilizers is not endorsed. Due to the limited supply of soluble nutrients in organic production systems, nutrient deficiency may limit plant growth and yield. The sole use of peat for pot-based cultures is also discouraged in organic production systems. Therefore, viable alternatives for highly soluble fertilizers and pure peat substrates have been studied using leek [*Allium ampeloprasum* L. var. *Porrum]* as a test plant. Plants were grown on peat-based substrates with different rates of compost additions, and with and without inoculation with arbuscular mycorrhizal (AM) fungi. Inoculation with a commercial AM fungus inoculum resulted in colonization rates of up to 70% of total root length, whereas not inoculated plants remained free of root colonization. Mycorrhizal fungus colonization increased shoot Zn and K concentrations, but did not significantly affect shoot dry matter or shoot N and P concentrations. In contrast, compost addition increased plant growth, and also increased P and K concentrations in plants. I conclude that plants with high rates of mycorrhizal colonization can be obtained on peat-based substrates, but that under these conditions plants may not consistently benefit in growth from the mycorrhizal symbiosis. In contrast, additions of compost are a possible means to improve the substrate quality in organic horticultural production.

2.2 Introduction

Peat-based substrates are widely used in horticulture to produce seedlings for outplanting or to grow commercial crops. These substrates are usually supplemented with soluble fertilizers in conventional production systems to achieve optimal supply of nutrients such as N and P.

The use of synthetic chemical fertilizers is discouraged in organic horticulture. The activity of soil microorganisms should contribute to the mobilization of mineral nutrients in the soil (Herrmann and Plakolm, 91). It is sometimes assumed that conventional methods of applying highly soluble nutrients in combination with pesticides may have a negative effect on plant quality for human consumption (Asami et al., 03).

The use of peat is also critically viewed for other reasons by organic growers. Peat is a limited natural resource and use of peat at the present rates is not sustainable (George and Eghbal, 03; Joosten and Clarke, 02).

Official guidelines for organic growers, presented e.g. by the European Union (04) and organic growers associations in many countries, mandate the use of organic or non-soluble fertilizers and a reduction of peat amendments to growth substrates to a maximum of 70% in the next few years (George and Eghbal, 03). This results in problems for producers, because many vegetable and ornamental plants have a high nutrient demand for satisfactory growth and yield. In addition, often only high quality vegetable products or ornamental plants without any deficiency symptoms can be marketed.

For the long-term economic success of ecological greenhouse horticulture, it is therefore important (a) to reduce the amount of peat in pot cultures without loss of plant quality, and (b) to define methods to improve nutrient supply from organic sources.

Various substitute materials have been tested to replace peat at least partly in growth substrates. Such substitute materials can consist of bark, coconut residues (Linderman and Davis, 03b), other bio-solids (Ozores-Hampton et al., 99), or compost (Veeken et al., 04). Compost has been widely used in traditional agriculture and horticulture and has beneficial effects, for example, on soil structure or soil biota (Carpenter-Boggs et al., 00; Wells et al., 00). Compost applications were avoided in many modern greenhouse horticultural systems due to a risk of transmitting plant diseases with compost applications. However, high quality composts, e.g. produced from organic household waste, can be almost free of pathogenic micro-organisms and may even have a suppressive effect on soil born diseases (Schüler et al., 89). High quality composts also have a high nutrient content. A substrate of 20% high quality compost mixed with peat is therefore recommended for current practice of organic horticulture in Germany and Switzerland (George and Eghbal, 03).

An improvement of the plant nutrient status in organic operations may require the application not only of composts, but also of other organic fertilizers. In addition, a "living" component, *i.e.* rhizosphere or soil micro-organisms, may help the plants to mobilize and acquire nutrients from the substrate. A group of soil micro-organisms that live in very intimate contact with the root are the arbuscular mycorrhizal (AM) fungi. These fungi are known to assist the plant in the uptake of nutrients and to improve plant growth (Douds et al., 05), including growth of *Allium* species (Dickson et al., 99), on soils low in phosphorus (P). They occur both in natural ecosystems and in agricultural soils (Smith and Read, 1997). AM fungus colonization often leads to increased

plant uptake of P, nitrogen (N), zinc (Zn), and copper (Cu), and sometimes also of potassium (K) (George, 00). Phosphate from organic fertilizers may be particularly accessible to AM colonized plants (Linderman and Davis, 04). In addition, much published evidence shows disease suppression in plants due to colonization by AM fungi (Kasiamdari et al., 02). Mycorrhizal fungi can also stabilize soil aggregates (Piotrowski et al., 04), and some reports show that mycorrhizal plants may be more resistant to stresses such as drought (Neumann and George, 04) or salinity (Tian et al., 04). Plant phytohormone levels can also be affected by mycorrhizal fungus colonization (Shaul-Keinan et al., 02).

Only a few studies have investigated the effect of compost supplements on mycorrhizal and non-mycorrhizal plant seedlings. Substrates with composts may be adequate for mycorrhizal plants (Goswani and Jamaluddin, 01; Linderman and Davis, 01), if the quality of the compost is sufficient (Boddington and Dodd, 00; Raviv et al., 98). Sáinz et al. (98), however, pointed out that compost additions may reduce mycorrhizal root length colonization and therefore the activity of AM fungi. Thus, at present it is not clear whether compost additions and mycorrhizal fungus inoculation are complementary measures to increase yield and yield stability in organic operations.

Therefore, I utilized leek as a test plant in two experiments studying whether (a) commercial or specifically prepared peat-based substrates support AM fungus colonization of plants, (b) AM fungus colonization is beneficial to plants on these substrates, and (c) compost additions affect the contribution of AM fungi to plant growth. The aim was to increase the understanding of the role of AM fungi in plant growth on organic substrates, and to advise producers on optimal compost and AM fungi addition treatments.

2.3 Material and Methods

2.3.1 Overview on experimental design and cultivation

Seeds of leek (Allium ampeloprasum L. var. Porrum ‘Prelina’) were placed in a commercial potting mix (KKS Bio-Potgrond, Klasmann-Deilmann GmbH, Geeste-Gross Hesepe, Germany) and kept for four weeks in trays placed in a greenhouse to allow germination and early plant growth. The trays were irrigated by hand to maintain optimal moisture conditions. Seedlings were then transplanted to 250-ml pots with two seedlings per pot. In Experiment 1, a commercial potting substrate was used. In Experiment 2, two substrates with different addition rates of compost (20% compost;

40% compost) were used (see below). The substrates were inoculated with one of three different types of AM fungus inocula (Pla, Bio, Tri) or remained without AM fungi (NAM). Five replications were used for each treatment in both experiments. The first experiment was placed in a greenhouse, the second experiment in a climate chamber.

Drip irrigation (40 $ml \cdot min^{-1}$) was used once a day in the climate chamber (total of 40 ml) and twice a day in the greenhouse (total of 40 to 80 ml depending on weather conditions) to maintain favorable water conditions in the substrate. Once a week the substrate was soaked with water to equalize the water content of the pots. The pots were standing on saucers and nutrient loss through leaching was prevented. Experiment 1 was carried out from 30 Aug. to 23 Oct. 2002 in a greenhouse facility at Großbeeren (long. 13°20´E; lat. 51°22´N), Germany. Average air temperatures in the greenhouse during this time were 21 to 24 (max. 30 °C) day/17 to 20 °C night and relative humidity was on average 70%. For experiment 2, a climate chamber was used with a light period of 16 h day/8 h night, a temperature of 22 C° day/18 °C night, and a relative humidity of 70% day/80% night. Light intensity provided by lamps (Agro Son T 400, Phillips, Hamburg, Germany) was between 450 and 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at different positions in the chamber. Pots were re-arranged in regular intervals in both experiments. Pots were always arranged in a completely randomised design.

2.3.2 Substrate preparation and characterization

All substrates used in this study were suitable for organic production. In Experiment 1, a commercial substrate (KKS Bio-Potgrond, Klasmann-Deilmann GmbH, Geeste-Gross Hesepe, Germany) was used that contained 80% v/v sphagnum peat (black peat) and 20 % v/v compost of green residues. The substrate also contained clay material, lime, horn meal and Thomas phosphate. This substrate is commonly used by organic growers in Germany. The extractable nutrients (extraction by $CaCl_2$ [N] and CAL [P, K]; information from the supplier) in this substrate were for N at 300-400 $mg \cdot L^{-1}$, P at 109-153 $mg \cdot L^{-1}$, and K at 290-415 $mg \cdot L^{-1}$. The substrate had a salt content of 1-2 $g \cdot L^{-1}$ and had a pH ($CaCl_2$) of 5-6.

In Experiment 2, the effect of increased compost additions to peat were tested. The compost was prepared from yard waste, shredded trees and bushes (Bruns, 98; Bruns and Schüler, 00). The material used had a wide C/N ratio (40:1) at the beginning of the composting process. After three months of composting extractable nutrient content in the compost was for N at 26 $mg \cdot L^{-1}$, for P at 335 $mg \cdot L^{-1}$, and for K at 1736 $mg \cdot L^{-1}$ (extraction by $CaCl_2$ [N] and CAL [P, K]; C. Bruns, personal communication). The substrate had a salt content of 2.8 $g \cdot L^{-1}$ and had a pH ($CaCl_2$) of 6.9. The compost was mixed with

sphagnum peat from the Baltic region (white peat) to obtain a compost substrate with 20% and 40% compost by volume. The substrates were limed with CaO to a pH of 6.2 and sieved to 5 mm. The compost substrate was of similar or higher horticultural quality as the commercial substrate (KKS Bio-Potgrond) used in Experiment 1.

Addition of 20% compost supplemented N at 5 $mg \cdot L^{-1}$, P at 67 $mg \cdot L^{-1}$, and K at 347 $mg \cdot L^{-1}$ to the substrate (C. Bruns, personal communication). In addition, N fertilizer was added to the substrate one day before the start of the experiment. The N fertilizer (a mixture of 33% horn meal 0-2 mm, containing 10% N, and 66% horn meal 2-6 mm, containing 14% N) was uniformly mixed into the substrate (7600 $mg \cdot L^{-1}$). Previous experience (C. Bruns, personal communication) suggests that two weeks after planting 25% of the added N was available to the plants, and that eight weeks after planting 85% of the added N was available. Therefore, the plant available N content of the compost substrate together with the horn meal fertilizer added up to 200 $mg \cdot L^{-1}$ (50 mg/pot) in the first two weeks after planting. The 40% compost substrate was fertilized with less N fertilizer (7400 $mg \cdot L^{-1}$), to account for the higher input of nutrients by the increased compost addition.

2.3.3 Water-holding capacity

The maximum water-holding capacities of all the substrates were evaluated following the method of Schaller (88): 50 g of the substrate was filled into a glass tube that was closed with fine gauze, and left soaking in water over night to absorb water through capillary rise. Shortly before the end of incubation time, water was raised in the surrounding vessel until water was visible at the soil surface. The surplus water dripped out when the tubes were allowed to stand on moist sand, allowing for the measurement of the maximum water-holding capacity (WC in g). The maximum water-holding capacity (WC in %) was calculated with the weight of the dried substrate (DW in g) (105 °C for 12h): $WC\ \% = 100 \times (WC\ g) \times (DW\ g)^{-1}$. The maximum water-holding capacities were 480, 420 and 550% in the commercial substrate (KKS), the 20% compost substrate and the 40% compost substrate, respectively.

2.3.4 Inoculation with AM fungi

Inoculation with AM fungi in Experiment 1 was carried out with one of three different commercially available inocula: Pla (TerraVital Hortimix with *G. mosseae, G. intraradices, G. claroideum* and *G. microaggregatum*, >50 infective units per ml inoculum; Plantworks Ltd., Heeley Close, Sittingbourne, Kent, UK), Bio (Endorize-Mix with *G. mosseae, G. intraradices, Glomus sp*., infective units not specified; Biorize, Rue Sainte Anne, Dijon, France), and Tri (*G. mosseae*, *Glomus intraradices,* and *G. etunicatum*, 50 infective units per ml inoculum; Triton, AMykor GmbH, Wolfen, Germany). Inocula

were mixed uniformly into the potting substrate before planting the seedlings. Addition rates were used according to the suppliers' recommendation and were Pla 5% v/v, Bio 5% v/v, and Tri 3% v/v. The same inocula were used in Experiment 2. Non-mycorrhizal (NAM) treatments were supplied with autoclaved (121 °C for 20 min) Pla inoculum. In addition, a filtrate (589/3 blue ribbon paper filter, Schleicher & Schuell Bioscience GmbH, Dassel, Germany) of non-sterilized Pla inoculum also was added to NAM pots in an effort to supply similar amounts of nutrients and micro-organisms other than AM fungi to all treatments.

2.3.5 Harvest and plant analysis

Both experiments ended eight weeks after planting. Shoots were separated from the roots, fresh weight (FW) recorded, washed and dried at 80 °C for two days, and dry weight (DW) also recorded. The shoots were ground in a centrifugal grinder using a 0.25 mm sieve.

The roots were washed and separated from the substrate with running cold water using a set of sieves (smallest sieve size 1 mm). The FW and DW were recorded and a representative sub sample for assessment of mycorrhizal fungus colonization was taken and stored in 10% isopropanol.

Shoot samples were dry ashed and dissolved in 18.5% HCl. Potassium, Zn, and Cu (Experiment 2 only) were analyzed with an atomic absorption spectrophotometer (Perkin Elmer 3300, Überlingen, Germany) and P photometrically with an EPOS-Analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar Vario EL, Hanau, Germany).

Mycorrhizal fungus colonization of roots was determined following the method of Koske and Gemma (89) with slight modifications. Roots were cleared with 10% KOH, acidified with 2N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization was determined with a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 50x using the grid line intersection method (Giovannetti and Mosse, 80).

2.3.6 Statistics

Data in Experiment 1 were subjected to a one-way analysis of variance, with inoculum type as treatment levels (n = 5). Mean separation was carried out with the Newman-Keuls method ($P < 0.05$). In Experiment 2, data were analyzed by a two-factorial analysis of variance, with compost addition rates and mycorrhizal inoculation as experimental factors (n = 5). Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, U.S.) software.

2.4 Results

2.4.1 Experiment 1

Roots were colonized by AM fungi in treatments with live mycorrhizal inoculum (Fig. 6). The percentages of colonized root length in AM plants were between 20 and 30%, but were not significantly different between the three different mycorrhizal inocula. The treatment without live mycorrhizal inoculum (NAM) remained free of mycorrhizal root colonization.

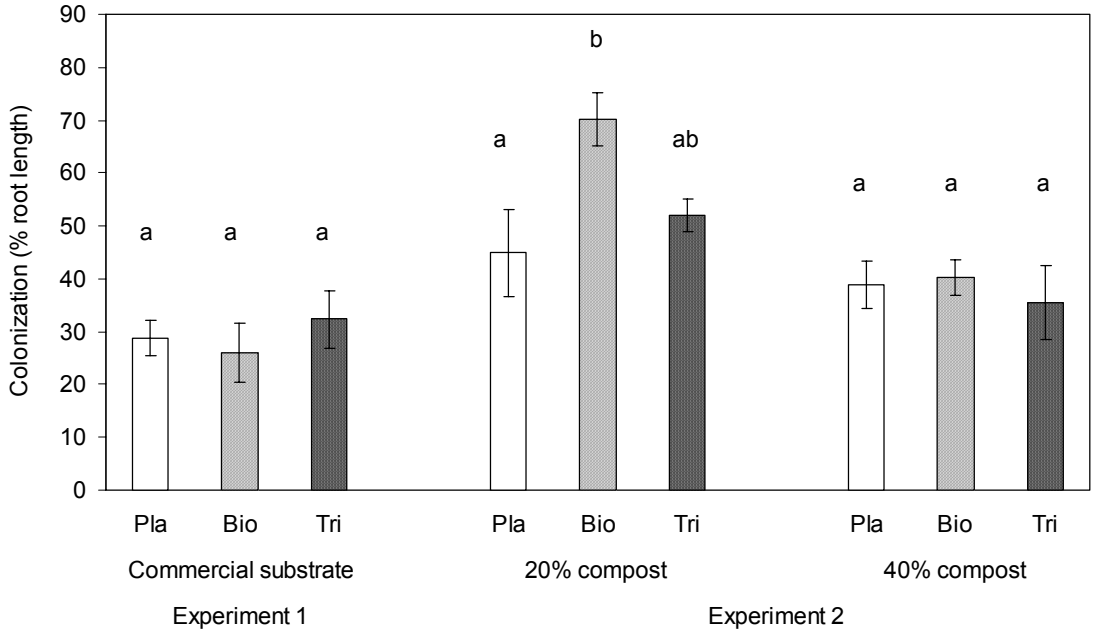


Fig. 6: Percentage root length of leek plants colonized by AM fungi eight weeks after planting on commercial growing substrate (Experiment 1; left) or in compost-peat substrates (Experiment 2; center and right). In both experiments, plants were either non-inoculated with mycorrhizal fungi or were inoculated with one of three mycorrhizal inocula (Pla, Bio, Tri). Differences between Pla, Bio, and Tri treatments within each experiment were tested with a Newman-Keuls test ($P<0.05$). Different letters denote significantly different means; means of 5 observations ± SE (T).

Shoot (Tab. 1) and root (data not shown) dry weights were not significantly affected by the inoculation treatments. Similarly, AM fungus root colonization had no significant effect on shoot N, P, and Zn concentrations. In contrast, K shoot concentrations were increased in mycorrhizal plants (Tab. 1). The highest K concentration was measured in shoots of the Pla treatment.

Tab. 1: *Experiment 1* (commercial substrate). Shoot dry weight (DW) and shoot element (N, P, K, and Zn) concentrations of leek plants eight weeks after planting. Plants were either non-inoculated with mycorrhizal fungi (NAM), or were inoculated with one of three mycorrhizal inocula (Pla, Bio, Tri). Effects of the treatment (mycorrhizal inoculation (m)) were tested with a one-way ANOVA. Different letters denote significant differences between means as determined by the Student-Newman-Keuls test ($P<0.05$). Values are means of 5 observations ± SE.

	DW	Element concentration			
	g pot^{-1}	g [kg DW]$^{-1}$			mg [kg DW]$^{-1}$
		N	P	K	Zn
NAM	1.1 ± 0.1	13.0 ± 0.3	2.9 ± 0.1	16.3 ± 1.2a	32.2 ± 2.6
Pla	1.2 ± 0.0	13.6 ± 0.6	2.7 ± 0.1	35.8 ± 2.1c	38.4 ± 2.3
Bio	0.9 ± 0.1	12.2 ± 0.5	3.1 ± 0.0	25.9 ± 0.6b	35.4 ± 1.6
Tri	1.0 ± 0.2	14.1 ± 0.5	3.2 ± 0.2	23.8 ± 1.6b	38.4 ± 3.9
P (m)	0.225	*0.070*	*0.104*	*<0.001*	0.350

2.4.2 Experiment 2

The percentage root length colonization with AM fungi in Experiment 2 was higher (t-test; $P< 0.05$) in 20% compost than in 40% compost (Fig. 6). Highest root colonization rates were observed in 20% compost, in the Bio treatment. However, root colonization was not significantly different between the three live mycorrhizal inocula in 40% compost. As in Experiment 1, NAM plant roots remained free of AM fungi.

Shoot (Tab. 2) and root (data not shown) dry weights were not significantly affected by the treatments. Shoot dry weight was much higher in Experiment 2 (Tab. 2) than in Experiment 1 (Tab. 1). Shoot N, Zn and Cu concentrations were not significantly affected by the compost treatments. Shoot P and K concentrations were increased in the 40% compost treatment compared to the 20% compost treatment. Shoot Zn concentrations were significantly increased in mycorrhizal compared to non-mycorrhizal plants at 20% compost supply (Tab. 2). At 40% compost supply, shoot of plants in the Bio treatment had the highest Zn and Cu concentrations.

Tab. 2: *Experiment 2* (compost addition rate) Shoot dry weight (DW) and shoot element (N, P, K, Zn and Cu) concentrations of leek plants eight weeks after planting. Plants were grown on compost-peat substrate with 20% compost or 40% compost, and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with one of three mycorrhizal inocula (Pla, Bio, Tri). Effects of the treatments (compost addition rate (c); mycorrhizal inoculation (m)) were tested with a two-way ANOVA. Different letters denote significant differences between means within one level of compost addition rate as determined by the Student-Newman-Keuls test (P<0.05). Values are means of 5 observations ± SE.

	DW	Element concentration				
	g pot^{-1}	g [kg DW]$^{-1}$			mg [kgDW]$^{-1}$	
		N	P	K	Zn	Cu
20% compost						
NAM	5.6 ± 0.3	9.2 ± 0.3	0.9 ± 0.0	11.9 ± 0.5	14.2 ± 0.7a	2.3 ± 0.2a
Pla	5.3 ± 0.3	10.8 ± 0.7	0.9 ± 0.0	13.1 ± 0.7	21.4 ± 2.3b	2.4 ± 0.4a
Bio	5.8 ± 0.4	9.4 ± 0.7	1.0 ± 0.1	10.8 ± 0.4	23.2 ± 1.6b	2.8 ± 0.4a
Tri	6.0 ± 0.8	10.1 ± 0.6	1.1 ± 0.0	11.0 ± 1.5	26.4 ± 2.2b	2.2 ± 0.2a
40% compost						
NAM	5.1 ± 0.3	9.1 ± 0.6	1.5 ± 0.0	15.0 ± 0.7	14.8 ± 0.6a	2.0 ± 0.0a
Pla	7.2 ± 0.5	9.2 ± 0.6	1.5 ± 0.1	13.8 ± 0.4	16.4 ± 1.4a	2.0 ± 0.0a
Bio	5.5 ± 0.6	8.9 ± 0.7	1.6 ± 0.1	15.4 ± 1.0	25.0 ± 3.9b	3.0 ± 0.3b
Tri	7.0 ± 0.6	9.9 ± 0.6	1.4 ± 0.1	13.8 ± 1.0	19.2 ± 1.6ab	2.2 ± 0.2a
P (c)	0.157	0.174	*<0.001*	*<0.001*	0.100	0.549
P (m)	0.108	0.295	0.279	0.573	*<0.001*	*0.018*
P (c x m)	0.061	0.578	*0.024*	0.183	*0.102*	*0.669*

2.5 Discussion

Compost addition to peat can be a source of plant nutrients and at the same time contribute to the protection of global peat resources. Compost addition rates of 20% [v/v] to a peat based substrate are now in use for commercial substrates. The present results show that a compost addition rate of 40% can also be recommended. Plants had increased P and K uptake on these substrates, and plant element concentrations did not indicate any risk of toxicity. Compost used for this purpose must be low in salt content, and of course should also be free of contamination with heavy metals or organic toxins. Compared to standard values for leaves of *Allium cepa* (Bergmann, 93), element concentrations indicated deficient supply of N in both experiments; and low supply of P, Zn and K in Experiment 2. Low N concentrations of plants in both experiments (Tab. 1

and (Tab. 2) show that even relatively high compost additions and addition of horn meal at moderate rates cannot supply sufficient N to plants during periods of fast growth. Nitrogen nutrition of potted plants in ecological production systems remains problematic. Possible solutions include liquid organic N fertilizers (such as vinasse) and addition of organic N fertilizers to the substrate some time before planting.

The lower shoot dry weight in Experiment 1 compared to Experiment 2 was probably explained by sub-optimal growth conditions (high temperatures) in the greenhouse compared to the climate chamber. In contrast, N deficiency was less severe in Experiment 1 than in Experiment 2 (see Tab. 1 and Tab. 2) for shoot N concentrations, perhaps because compost and additional N (horn meal) was supplied to the plant substrate in Experiment 2 directly before the start of the experiment, whereas in Experiment 1 the commercial substrate was supplied with additional nutrients several months before the start of the experiment, so that more N from horn meal became available during this time.

All three test substrates did not support spontaneous mycorrhizal colonization of the leek plants. This indicates that the peat, but also the added compost contained no or very low amounts of infectious mycorrhizal material. Probably, the density of mycorrhizal propagules is low in certain types of green material used for compost preparation, and high temperatures during composting further reduce the number of live mycorrhizal propagules. It is likely, that this finding applies in general to peat-compost substrates. If producers plan to use mycorrhizal plants on organic potting substrates, for example because of superiority of mycorrhizal plants in disease resistance or flowering ability, the application of mycorrhizal inoculum is necessary.

All three commercial inocula used successfully colonized the roots. The extent of root colonization was different between the inocula only in Experiment 2 (20% compost), and this difference was not clearly related to different effects of the inocula on plant activity. For example, although Bio inoculum caused the highest colonization rate in this case, shoot Zn concentrations were not higher in Bio plants than in Pla or Tri plants. Thus, the present data indicate that the use of all three types of inocula can be recommended, but further tests with more inocula and under various environmental conditions are required to generalize this result.

Root colonization by Bio and Tri inocula was greater in 20% compost versus 40% compost (Fig. 6), very likely because of the lower level of nutrients (especially P) supplied in the 20% compost treatment versus 40%. Only the supply of N, but not of other

nutrients, was equilibrated between 20% and 40% compost addition rate. A decrease of colonization with increased supply of mineral nutrients, especially of P (Boddington and Dodd, 00; Douds and Reider, 03), or with addition of certain types of compost or peat (Linderman and Davis, 03a; Sáinz et al., 98; Wang et al., 93), has often been observed. It is also possible that the higher water capacity of the 40% compost substrate had some effect on mycorrhizal colonization. Some AM fungi show a lower hyphal growth in moist soils (Smith and Read, 97).

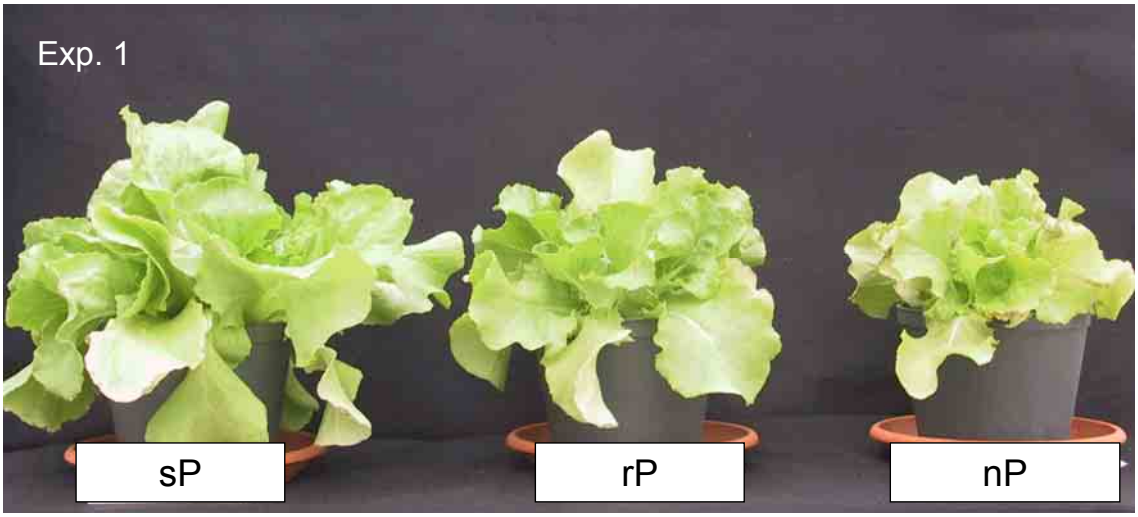
Under the conditions of both experiments, plant dry weight was not affected by mycorrhizal fungus colonization, although colonization rates were considerable. Decreases of shoot growth upon mycorrhizal colonization are sometimes observed, in particular at sub-optimal light conditions for plant growth (Smith and Gianinazzi-Pearson, 90). In the present experiments, light supply was probably sufficient to allow for carbon fixation to sustain carbon expenses for mycorrhizal fungus colonization without negative impact on plant growth. Increases in shoot growth upon mycorrhizal colonization are often observed on substrates with low nutrient availability, in particular on substrates with low P availability. In the present experiment, plants had low N shoot concentrations (Tab. 1 and Tab. 2) and probably were limited in growth mainly by low N availability in the substrate. AM fungus hyphae can transport N to the plant, but not to an extent that N deficiency of fast-growing plant species can be overcome (Hawkins and George, 99). It is surprising, however, that P concentrations were not increased in mycorrhizal plants compared to non-mycorrhizal counterparts. Most evidence of increased P uptake in mycorrhizal plants comes from experiments and observations on mineral soils. Some evidence indicates that also freshly applied organic P sources can be utilized by AM fungi (Feng et al., 03). However, it is possible that plant P uptake from organic substrates such as peat or compost is less dependent on AM fungus colonization than P uptake from soils with mineral P sources. Compost may contain P sources that are either readily accessible to plants, or are inaccessible to plants and AM fungi alike through physico-chemical fixation in form of condensed calcium phosphates such as apatites or octacalcium phosphates (Frossard et al., 02; Grey and Henry, 99). Alternatively, the AM fungi contained in the commercial inocula used in this study may be specifically adapted to P supply conditions in mineral soil.

Hyphae of AM fungi can transport not only N and P (George et al., 92), but also Cu (Li et al., 91), Zn and probably K (George, 00). This can lead to increased K and Zn concentrations in mycorrhizal plants. The present data confirm increased Zn uptake in mycorrhizal compared to non-mycorrhizal plants (Tab. 2) when the Zn status of non-mycorrhizal plants was relatively low (Experiment 2). When the Zn status of non-

mycorrhizal plants was higher (Experiment 1), the effect of AM fungi colonization on Zn uptake was less.

In conclusion this experiment indicated that (a) a compost addition rate of 40% in a peat based substrate can produce a growth substrate of high quality for ecological production, (b) peat-compost organic substrates did not contain live AM fungus propagules, (c) commercial inocula were used successfully to obtain high AM fungus colonization rates of potted plants, (d) AM fungus colonization can actively support plant Zn or K uptake on these substrates, and (e) plant P uptake and growth were not increased by AM colonization. Perhaps, P bound in substrates in organic form is less available to many AM fungi than P bound to soil minerals.

Chapter 3



3 Accessibility of phosphate to lettuce plants inoculated with arbuscular mycorrhizal species from different origins and grown on peat substrate with phosphate fertilizers of varying plant availability

3.1 Abstract

Rock phosphate in a fertilizer frequently used in organic horticulture. However, mineralization of rock phosphate can be too slow to meet the high nutrient demand of young plants. The use of beneficial soil microorganisms can contribute to plant nutrient uptake and growth. In this study, it was examined growth and N, P, K, Zn and Mg uptake of lettuce plants inoculated with different species of arbuscular mycorrhizal fungi and fertilized with either rock phosphate, highly soluble P, or without added P fertilizer, using cabbage lettuce [*Lactuca sativa L.* 'Nadine'] and loose-leaf lettuce [*Lactuca sativa L.* 'Smile'] as test plants. Plants were grown on peat substrates with and without arbuscular mycorrhiza (AM). Inoculation with AM from differently managed soils resulted in colonization rates of up to 65%. AM inoculation did not promote dry matter production of lettuce grown on substrates with low P availability. Although AM increased shoot N, Mg, and Zn concentrations, shoot P concentration was not increased. Differences in some parameters were observed between AM species, but not from the non-mycorrhizal control. Loose-leaf lettuce was best at utilizing P from rock phosphate, producing similar dry weight as with soluble P fertilizer. It is concluded that AM did not have a beneficial effect for lettuce plants with regards to P acquisition.

3.2 Introduction

Phosphorus (P) is an essential macronutrient involved in many physiological and biochemical processes, such as photosynthesis, synthesis of proteins and vitamins, or as a component of biomembranes. P is taken up from the soil solution as phosphate, and during periods of rapid growth crops may take up as much as 2.5 kg P_2O_5 per hectare-day (Johnston and Steén, 00). To maintain optimum crop productivity on soils with low P availability, crops need to be fertilized with readily plant available P in adequate amounts (Johnston and Steén, 00).

In organic horticulture, the only forms of P fertilizer that are allowed are organic material (e.g. chicken manure) and rock phosphate (European Union, 04). On soils with a pH above five, rock phosphate is a slowly soluble fertilizer (Steffens et al., 05; El Des-

sougi et al., 03) that usually produces a poorer plant growth response than do highly soluble chemical P fertilizers (Steffens et al., 05). Phosphate-solubilizing microorganisms might help to increase plant availability of rock phosphate, particularly in organic horticulture.

Arbuscular mycorrhizal (AM) fungi is a plant roots colonizing microorganism, that has often been observed in agricultural soils all over the world, generally with beneficial effects for the crop plants (Morgan et al., 05). Organic management has been reported to increase the biodiversity of AM fungi compared to conventionally-managed soils (Mäder et al., 02; Oehl et al., 04; 05). In addition, organic fertilization supports the development of AM fungi, whereas highly plant available mineral fertilization regimes, which are avoided in organic plant production, have been shown to inhibit AM fungi (05b; Gryndler et al., 05a). It can be hypothesized that some of the AM strains isolated from organically-managed soils may have special attributes that would increase the plant availability of nutrients from fertilizers used specifically in organic agriculture, e.g., rock phosphate or horn meal. As a result, these AM species may have a higher benefit for their symbiont and thus be reciprocally supported and favoured by selection.

AM has been reported to increase P uptake from rock phosphate in *Zea mays* L. grown on acidic soils (Alloush and Clark, 01) and in barley lacking root hairs (Chen et al., 05), and it has increased the yield of *Alfalfa* fertilized with rock phosphate (Barea et al., 02). In combination with organic matter or phosphate-solubilizing bacteria, AM fungi can be very efficient in exploiting rock phosphate (Barea et al., 75, 02; Duponnois et al., 05). Mechanisms for mobilization of P may include lowering of pH in the growth substrate (Li et al., 91; Son et al., 06), excretion of organic acids such as α-ketoglutaric acid (Duponnois et al., 05), or excretion of phosphatases. The excretion of phosphatases as a mobilization mechanism for hydrolyzing organic phosphates is similar for AM fungi and plants (Joner and Johansen, 00, Koide and Kabir, 00), but acidic phosphatases may also solubilize rock phosphate. Phosphorus is the least mobile macronutrient in soil, because it is readily sorbed (e.g., to metal oxides), occurs in mineral forms of low solubility (e.g., calcium phosphates such as apatite), and can be incorporated into organic molecules which are poorly availabile to plants (Marschner, 95; Frossard et al., 02). The advantage of mycorrhizal roots compared to non-mycorrhizal roots is that the hyphae exploit a higher soil volume than the root by penetrating soil pores that are not reachable by plant roots (Drew et al., 03). AM hyphae increase the absorptive surface area of the root and take up mobilized P quickly before it is bound again.

It is hypothesized that (a) AM fungi inoculation will increase the dry weight and nutrient uptake of plants grown on substrates with low P supply due either to low P fertilization or to low solubility of applied rock phosphate fertilizer, (b) AM strains originat-

ing from organically-managed or natural habitats will be superior in the mobilization of P from rock phosphate compared to a non-organically propagated commercial inoculum (Exp. 2), (c) soluble P fertilizer will produce the highest shoot dry weight and shoot P concentration and will suppress root length colonization by AM, compared to rock phosphate and low P fertilization, and (d) slowly-soluble rock phosphate fertilizer will provide a longer-lasting source of P fertilization than highly-soluble phosphate fertilizer, and thus it's beneficial effects will become more evident with time.

The aim of this research was to investigate the effect of arbuscular mycorrhizal fungi on growth and nutrient uptake of lettuce grown on a peat substrate fertilized with either rock phosphate, soluble P, or without added P fertilizer.

Three different AM inocula, one isolated from an organic-dynamically managed soil, one from a nature conservation area, and one that was commercially propagated, were tested for their effect on shoot dry weight production, shoot N, P, K, Zn, and Cu concentrations, and root colonization rate of two lettuce varieties.

3.3 Materials and Methods

3.3.1 Overview of experimental design and cultivation

In Experiment 1, seeds of cabbage lettuce (Lactuca sativa L. 'Nadine', Rijk Zwaan) were germinated directly in 500 ml pots filled with a commercial white peat substrate containing particles of volcanic clay (Fruhstorfer Erde, Hawita Gruppe, Vechta, Germany) in a dark climate chamber at 10 °C and 80% humidity for one week. Two seedlings per pot (n=5) were grown for 8 weeks in a greenhouse facility at Großbeeren, Germany (long. 13°19´60´E; lat. 51°22´0´N), during the summer of 2003. Average air temperature in the greenhouse during this time was 23 °C (min 11 °C and max. 36 °C) during the day and 17 °C (min. 11 °C and max. 29 °C) at night. Relative humidity was on average 69% during the day and 83% at night. The daily (14 h) mean light intensity (PAR) was 17.6 $mol \cdot m^{-2}$ (max. 674 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The plants were irrigated by hand with distilled water to maintain optimum substrate moisture for plant growth. The soil moisture at the beginning of the experiment was 60%. Every second or third day the pots were weighed and the water content of the substrate was equalized.

In Experiment 2, seeds of loose-leaf lettuce (*Lactuca sativa L.* 'Smile', Bruno Nebelung GmbH & Co.) were germinated in a dark climate chamber at 10 °C and 80% humidity and transferred to separate 750-ml pots filled with a commercial white peat substrate with clay (Terreau Universal Gepac, Einheitserde + Humuswerk, Sinntal-Jossa, Germany). Two seedlings per pot (n=4) were grown during the summer of 2004 for 10

weeks in a greenhouse facility at Großbeeren. The average air temperature in the greenhouse during this time was 21 °C (min. 15 °C and max. 31 °C) during the day and 20 °C (min. 15 °C and max. 32 °C) at night. Relative humidity was on average during the day 61% and at night 64%. The daily (14 h) mean light intensity (PAR) was 13.0 $mol \cdot m^{-2}$ (max. 1337 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during the day. The plants were irrigated each day by hand with 60 to 120 ml distilled water to maintain optimum substrate moisture for plant growth.

The pots allowed free drainage from the bottom. Leachate was collected for each pot using a saucer, and was returned to the substrate to prevent loss of nutrients. The pots were arranged in a completely randomized design, and were re-arranged at regular intervals.

3.3.2 Substrate preparation

Three different P fertilization treatments were used: no added P (nP), rock phosphate (rP), and soluble P (sP).

In the first experiment, the extractable nutrients before fertilization (extraction by $CaCl_2$ [N] and CAL [P, K]; analysed by laboratory of the supplier) in the substrate were ($mg \cdot L^{-1}$): N, 24; P, 3; and K, 12; with a salt (mainly KCl) concentration of 0.34 $g \cdot L^{-1}$ and a pH ($CaCl_2$) of 5.8. The nP treatment was fertilized with K_2SO_4 at 178 $mg \cdot L^{-1}$ to provide equal amounts of K. The rP treatment was fertilized with rock phosphate (P_2O_5 31%) at 493 $mg \cdot L^{-1}$, and the sP treatment with KH_2PO_4 at 280 $mg \cdot L^{-1}$, for a P addition rate of 64 $mg \cdot L^{-1}$ in these two treatments. In addition, N fertilizer was added to the substrate one day before the start of the experiment. The N fertilizer (a mixture of 33% horn meal 0-2 mm, containing 10% N, and 66% horn meal 2-6 mm, containing 14% N) was uniformly mixed with the substrate (horn meal at 6670 $mg \cdot L^{-1}$). Previous experience (C. Bruns, personal communication) indicated that two weeks after planting, 25% of the added N was available to the plants, and that eight weeks after planting, 85% of the added N was available. Under this assumption, the plant available N content of the commercial peat substrate together with the horn meal fertilizer added N was 200 $mg \cdot L^{-1}$ in the first two weeks after planting. After two weeks of growth each pot was additionally fertilized with 40 ml of 5% vinasse solution, containing N at 120 $mg \cdot pot^{-1}$, because of N deficiency symptoms. The substrate was also fertilized with K^+ at 400 $mg \cdot L^{-1}$ and Mg^{2+} at 80$mg \cdot L^{-1}$ (30-10 K_2O – MgO), Fe^{2+} at 1.5 $mg \cdot L^{-1}$ (Fe-Chelat DTPA 6%), Zn^{2+} at 7.7 $mg \cdot L^{-1}$ ($ZnSO_4$), and Cu^{2+} at 7.7 $mg \cdot L^{-1}$ ($CuSO_4$).

In the second experiment, the extractable nutrients before fertilization (extraction by $CaCl_2$ [N] and CAL [P, K]; analysed by laboratory of the IGZ) in the substrate were: N, 50 $mg \cdot L^{-1}$; P, 34 $mg \cdot L^{-}$; and K, 67 $mg \cdot L^{-1}$. The substrate had a salt concentration of <0.8 $mg \cdot L^{-1}$ and a pH ($CaCl_2$) of 6.0. The nP and rP treatments were fertilized with K_2SO_4 at

120 mg L^{-1}. The rP treatment was fertilized with rock phosphate (P_2O_5 31%) at 331 mg·L-1, and the sP treatment with KH_2PO_4 at 188 mg·L^{-1}, for a P addition rate of 43 mg·L^{-1} in these two treatments. Nitrogen was also added as horn meal (horn meal at 7130 mg·L^{-1}). Seven weeks after planting each pot was additionally fertilized with 3 g horn meal (2-6 mm) because of N deficiency symptoms. Potassium was added at 279 mg·L^{-1}, Mg^{2+} at 80mg·L^{-1} (30-10 K_2O–MgO and $MgSO_4$), Fe^{2+} at 1.5 mg·L^{-1} (Fe-Chelat DTPA 6%), Zn^{2+} at 7.7 mg·L^{-1} ($ZnSO_4$), and Cu^{2+} at 7.7 mg·L^{-1} ($CuSO_4$). Initial nutrient concentrations of the substrates used in the two experiments were almost similar, with P at 67/77 mg·L^{-1}, K^+ at 492/400 mg·L^{-1}, Mg^{2+} at 80/80 mg·L^{-1}, and N at 824/930 mg·L^{-1}, respectively.

3.3.3 Inoculation with AM fungi

Inoculation with AM fungi in both experiments was carried out with one commercially available inoculum ('Pla') (TerraVital Hortimix with *G. mosseae, G. intraradices, G. claroideum* and *G. microaggregatum*, >50 infective units per ml inoculum; Plantworks Ltd., Heeley Close, Sittingbourne, Kent, UK). In experiment 2, two single strain inocula (isolated by F. Oehl, University of Basel, Switzerland) were used. The first one ('34'), from an organic-dynamically managed soil (*G. etunicatum*, ISCB 34, DOK experiment, Switzerland; Redecker, 06) and the other ('47') from a nature conservation area (*G. lamellosum*, ISCB 47, neglected grassland, Kaiserstuhl, Germany; Redecker, 06). The inocula were mixed uniformly (5% v/v) into the potting substrate before the seedlings were planted. Non-mycorrhizal (NAM) treatments were supplied with autoclaved (121°C for 20 min) Pla inoculum. In addition, a filtrate (589/3 blue ribbon paper filter, Schleicher & Schuell Bioscience GmbH, Dassel, Germany) of non-sterilized Pla inoculum was also added to NAM pots to establish a similar microflora in Pla and NAM treatments.

3.3.4 Harvest and plant analysis

The cabbage lettuce was cut above the soil surface. The loose-leaf lettuce was harvested the first time after seven weeks of growth, leaving 3 to 5 leaves on the plant, and three weeks later the whole shoot was cut above the soil surface. Shoot fresh weight (FW) was recorded and, after drying for two days at 80°C, dry weight (DW) was also recorded. The shoots were ground in a centrifugal grinder using a 0.25-mm sieve. Shoot samples were dry ashed and dissolved in 18.5% HCl. Potassium, Zn, and Cu were analyzed with an atomic absorption spectrophotometer (Perkin Elmer 3300, Überlingen, Germany) and P and Mg were measured photometrically with an EPOS-Analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar Vario EL, Hanau, Germany).

For the investigation of AM fungal root length colonization, representative samples were taken from the contents of the planting pots, and roots were washed free from the peat substrate using a set of sieves (smallest sieve size 1 mm). The root samples were stored in 10% isopropanol. Mycorrhizal fungus colonization of roots was determined following the method of Koske and Gemma (Koske and Gemma, 89) with slight modifications. Roots were cleared with 10% KOH, acidified with 2N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization was determined with a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 100x using the grid line intersection method (Giovannetti and Mosse, 80).

3.3.5 Statistics

The data were analyzed by a two-factorial analysis of variance, with phosphorus fertilization treatments and mycorrhizal inoculation as experimental factors (Exp. 1, n = 5; Exp. 2, n = 4). If variance homogeneity was not present an analysis of variance was used with the SAS procedure MIXED in consideration of possible heterogeneity of variance (i.e. in dependency of the feature variability it was worked with a joint sample variance or with different sample variances within each fertilization treatment and/or within each mycorrhizal inoculation treatment). Mean separation was carried out with the Tukey test ($p < 0.05$). For the second harvest the Dunnett T3 test was used in case of heterogeneity of variance. Root length colonization rate was analyzed with a Kruskal-Wallis Test. Data were analyzed using SPSS 13.0 (Chicago, Illinois, U.S.) and SAS 9.1 (SAS Institute GmbH, Heidelberg, Germany) software.

3.4 Results

3.4.1 Mycorrhizal colonization

In the first experiment, the root length colonization rate in treatments with live mycorrhizal inoculum averaged 24.3 ± 7.5%. The percentage of colonized root length in AM plants in the different P treatments did not differ significantly (nP at 18.0 ± 9.8%; rP at 11.8 ± 7.3%; sP at 6.7 ± 6.5). In the second experiment, the root length colonization rate in treatments with live mycorrhizal inoculum averaged 65.2 ± 2.2%. The percentage of colonized root length in AM plants in the different P treatments did not differ significantly (nP at 53.2 ± 5.9%; rP at 73.8 ± 8.1%; sP at 66.7 ± 3.6). In both experiments the treatment without live mycorrhizal inoculum (NAM) remained free of mycorrhizal root colonization.

3.4.2 Shoot dry weight, nutrient concentration and content

Shoot dry weight. In experiment 1, shoot dry weight was significantly affected by P fertilization treatment, increasing in the order nP < rP < sP (Tab. 3). In contrast, shoot dry weight in the first harvest (7 weeks) of the second experiment was similar in the rP and sP treatments, which both had significantly higher values compared to the nP treatment (Tab. 4). At the second harvest (10 weeks), plants in the rP treatment had the highest shoot dry weight, followed by the sP treatment and then by the nP treatment. The second harvest included leaves not harvested in the first harvest as well as leaves formed during the intervening three weeks between harvests.

Tab. 3: *Experiment 1* Shoot dry weight (DW) and shoot nutrient (N, P, K, Zn and Cu) concentrations and N content of cabbage lettuce plants eight weeks after planting. Plants were grown on three P fertilization treatments (non P (nP), rock phosphate rP, soluble P (sP)), and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with a commercial mycorrhizal inocula (Pla). Effects of the treatments (P fertilization (f); mycorrhizal inoculation (m)) were tested with a two-way ANOVA. Different letters denote significant differences between means within one main factor as determined by the Tukey- test (P<0.05). Values are means of 5 observations ± standard error of the mean (SE). Cu concentrations were not significantly different and on average 3.73 ± 0.45 mg kg^{-1}.

	DW	Element concentration				Content
	g pot^{-1}	g [kg DW]$^{-1}$			mg [kg DW]$^{-1}$	mg pot^{-1}
		N	P	K	Zn	N
NAM	5.9 ± 1.3	34 ± 4a	1.7 ± 0.2	47 ± 2	63.9 ± 4.6a	179 ± 11a
Pla	5.9 ± 1.3	37 ± 5b	1.7 ± 0.2	45 ± 3	68.1 ± 5.5b	195 ± 14b
nP	3.6 ± 0.1a	45 ± 2c	1.2 ± 0.0a	52 ± 1b	6.7 ± 2.7c	160 ± 5a
rP	5.5 ± 0.1b	37 ± 3b	1.9 ± 0.1b	47 ± 3b	68.1 ± 3.0b	196 ± 8b
sP	8.5 ± 0.0c	24 ± 2a	1.9 ± 0.1b	40 ± 1a	53.3 ± 2.0a	205 ± 13b
P (f)	*<0.001*	*0.003*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
P (m)	0.942	*<0.001*	0.102	0.355	*0.046*	*0.021*
P (f x m)	0.590	0.878	0.153	0.492	0.392	0.337

In both experiments, inoculation with live mycorrhizal fungi did not increase shoot dry weight significantly compared to the non-mycorrhizal control (Tab. 3 and Tab. 4). In the first harvest of the second experiment, the 'Pla' treatment had the highest shoot dry weight over all P fertilization treatments and had significantly higher DW than that of treatment '47'. At the second harvest, this effect was maintained only in the rP treatment (Tab. 4). At the second harvest, the mean shoot dry weight of treatment '34' was highest in all P fertilization treatments except for rP, although differences were not significant, because of the high variance of the data. At the second harvest, plants of treatment '47' had the highest shoot dry weight on the sP treatment, whereas for all

other mycorrhizal treatments, shoot dry weight at the second harvest was highest when plants received rock phosphate.

Tab. 4: *Experiment 2* Shoot dry weight (DW) after 7 and 10 weeks and shoot nutrient (P, K, Mg and Zn) concentrations 7 weeks after planting of loose-leaf lettuce plants. Plants were grown on three P treatments (non P (nP), rock phosphate (rP), soluble P (sP)), and either non-inoculated with mycorrhizal fungi (NAM) or inoculated with mycorrhizal inocula (Pla, '34', '47'). Effects of the treatments (P fertilization (f); mycorrhiza inoculation (m)) were tested with a two-way ANOVA. Small letters denote significant differences between means within one P fertilization treatment and capital letters denote significant differences within one mycorrhizal treatment as determined by the Tukey-test (P<0.05). Values are means of 4 observations ± SE.

		7 weeks					10 weeks
		DW	Element concentration				DW
		g pot^{-1}	g [kg DW]$^{-1}$			mg [kg DW]$^{-1}$	g pot^{-1}
f	m		P	K	Mg	Zn	
nP	NAM	3.1 ± 0.3A	1.4 ± 0.1abA	69 ± 4B	7.1 ± 1.2ab	121 ± 2	12 ± 1A
	Pla	3.9 ± 0.7**A**	1.4 ± 0.2ab**A**	65 ± 11**A**	7.8 ± 1.4ab	117 ± 2	9 ± 3**A**
	'34'	3.1 ± 0.1*A*	1.7 ± 0.0b*A*	69 ± 2*B*	10.6 ± 0.3b*B*	147 ± 6*B*	18 ± 2*A*
	'47'	3.4 ± 0.4A	1.1 ± 0.1aA	67 ± 7B	4.1 ± 0.8a	95 ± 2	13 ± 2A
rP	NAM	4.9 ± 0.0abB	2.8 ± 0.6AB	40 ± 2A	6.6 ± 2.0	100 ± 1	43 ± 2bB
	Pla	5.3 ± 0.3b**AB**	1.9 ± 0.2**AB**	35 ± 1**A**	5.8 ± 0.5	98 ± 5	44 ± 1b**B**
	'34'	4.9 ± 0.1ab*B*	1.9 ± 0.3*A*	37 ± 1*A*	7.2 ± 1.8*AB*	116 ± 2*AB*	44 ± 4ab*B*
	'47'	4.2 ± 0.4aAB	1.7 ± 0.2A	29 ± 3A	3.2 ± 0.5	67 ± 7	29 ± 1aB
sP	NAM	5.4 ± 0.2abB	4.0 ± 0.2B	39 ± 2abA	5.0 ± 0.5	61 ± 5	24 ± 11AB
	Pla	5.9 ± 0.2b**B**	3.3 ± 0.4**B**	37 ± 2ab**A**	5.5 ± 0.5	85 ± 9	26 ± 10A**B**
	'34'	5.2 ± 0.3ab*B*	3.7 ± 0.1*B*	40 ± 1b*A*	5.6 ± 0.1*A*	67 ± 1*A*	37 ± 3*B*
	'47'	4.7 ± 0.1aB	3.2 ± 0.1B	31 ± 1aA	5.1 ± 0.6	64 ± 5	36 ± 1B
P (f)		*<0.001*	*<0.001*	*<0.001*	*0.011*	*<0.001*	*<0.001*
P (m)		*0.009*	*0.025*	0.210	*0.002*	*0.015*	0.064
P (f x m)		0.689	0.287	0.777	*0.009*	*0.035*	*0.014*

Shoot nutrient concentration. In the first experiment, shoot P concentration was equally high in the rP and sP treatments, but significantly lower in the nP treatment. Shoot N and Zn concentrations were contrariwise lowest in the sP treatment and highest in the rP treatment. Shoot K concentration was highest in the shoots of the nP and rP plants, but significantly lower in the sP treatment.

In the second experiment, statistical analysis of the main factor fertilization revealed that shoot P concentration increased significantly in the order nP < rP < sP. Within treatments '34' and '47', shoot P concentration was not significantly different between the nP and the rP plants (Tab. 4).

The main factor fertilization, revealed that shoot K concentrations of the rP and sP treatment were equal but significantly lower than the nP treatment (Tab. 4). Shoot Mg

and Zn concentrations between the three P fertilization treatments were not significantly different within the NAM, 'Pla' and '47' treatments. Only treatment '34' showed an effect similar to that seen in the first experiment, with lower shoot Mg and Zn concentrations in the sP treatment, compared to the nP treatment (Tab. 4).

In the first experiment, the 'Pla' treatment had no influence on shoot P concentration, but significantly increased shoot N and Zn concentrations (Tab. 3).

Comparing the mycorrhizal treatments over all P fertilization treatments in the second experiment, shoot P concentration was highest in the NAM and '34' treatments, although individual comparisons showed an effect only between treatments '34' and '47 in the nP treatment (Tab. 4). Statistical analysis of the main factor mycorrhiza showed that it had no significant effect on shoot K concentration, but individual comparisons within fertilization treatments showed that in the sP treatment, treatment '34' had a significantly higher shoot K concentration than that of treatment '47' (Tab. 4). Shoot Mg concentration was elevated in treatment '34' across all P fertilization treatments, but significantly higher only in comparison with treatment '47' in the nP treatment (Tab. 4). Individual comparisons of Zn concentration within each P fertilization treatment did not reveal any significant differences. In general, shoot Zn concentration was highest in treatment '34' on the nP and rP treatments, whereas in the sP treatment, treatment 'Pla' had the highest Zn concentration. Recapitulating the second experiment, it can be said that within the P fertilization treatments, shoot P, K, Mg, and Zn concentrations were almost always increased in treatment '34' and almost always decreased in treatment '47', but were never significantly different from the NAM treatment.

For the investigation of mycorrhizal performance on rock phosphate, nutrient concentrations of P, K, Mg and Zn were also analyzed after the second harvest (Tab. 5). The mycorrhizal treatments did not show any effect on shoot P or Zn concentrations. The NAM treatment had the highest shoot K concentration, followed by treatment 'Pla'. Treatment '47' had the lowest shoot K concentration. Shoot Mg concentration was significantly higher in treatments 'Pla' and '34' than in treatment '47' (Tab. 5).

Tab. 5: *Experiment 2.* Shoot nutrient (P, K, Mg and Zn) concentrations of loose-leaf lettuce plants 10 weeks after planting. Plants were grown on rock phosphate (rP) and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with a three mycorrhizal inocula (Pla, '34', '47'). Effect of the mycorrhizal treatment (m) was tested with a one-way ANOVA. Small letters denote significant differences between means as determined by the Dunnett T3-test ($P<0.05$). Values are means of 4 observations ± SE.

		Element concentration			
		g [kg DW]$^{-1}$			mg [kg DW]$^{-1}$
		P	K	Mg	Zn
rP	NAM	3.6 ± 0.6	14.5 ± 0.9c	6.6 ± 1.0ab	60 ± 5
	Pla	2.5 ± 0.2	12.3 ± 0.8bc	7.7 ± 0.5b	67 ± 5
	'34'	2.7 ± 0.3	10.7 ± 0.7ab	6.9 ± 0.3b	66 ± 3
	'47'	2.6 ± 0.0	8.7 ± 0.5a	4.6 ± 0.2a	56 ± 3
P (m)		0.153	*<0.001*	*0.014*	0.152

Shoot nutrient content. In the first experiment, shoot P, K and Zn content increased significantly in the order nP < rP < sP, but mycorrhiza had no significant influence (data not shown). Shoot N content was equally high in the rP and sP treatments and significantly higher than in the nP treatment (Tab. 3). The 'Pla' treatment increased N content significantly over that of the NAM treatment.

In contrast, in the second experiment only shoot P content in the NAM treatment was significantly increased by P fertilization treatment in the pattern nP < rP < sP (Tab. 6). Shoot P content of treatments 'Pla', '34', and '47' were similar in the nP and rP treatments but elevated in the sP treatment. Calculated over the two harvests (Tab. 7), the NAM treatment had the highest P content within the rP treatment, although the difference was not significant.

Shoot K content within mycorrhizal treatments '34' and '47', was significantly higher on the nP treatment compared to the rP treatment (Tab. 6). Analysis of the main factor P fertilization revealed that shoot Mg content was significantly higher in the sP treatment compared to the nP treatment, while that of the rP treatment was not significantly different from the other two. Within mycorrhizal treatments, shoot Zn content was significantly affected by P fertilization only within treatment '34', where it was higher in the rP treatment than in the sP treatment (Tab. 6).

Tab. 6: *Experiment 2.* Shoot nutrient (P, K, Mg and Zn) content of loose-leaf lettuce plants seven weeks after planting. Plants were grown on three P fertilization treatments (non P (nP), rock phosphate rP, soluble P (sP)), and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with a three mycorrhizal inocula (Pla, '34', '47'). Effects of the treatments (phosphate fertilization (f); mycorrhizal inoculation (m)) were tested with a two-way ANOVA. Small letters denote significant differences between means within one level of P fertilization treatment and capital letters denote significant differences within one mycorrhizal treatment as determined by the Tukey-test (P<0.05). Values are means of 4 observations ± SE.

		Element content			
		mg·pot^{-1}			µg·pot^{-1}
f	m	P	K	Mg	Zn
nP	NAM	4.0 ± 0.2abA	207 ± 11	21 ± 2ab	356 ± 16
	Pla	5.0 ± 0.5ab**A**	231 ± 22	28 ± 3bc	420 ± 39
	'34'	5.2 ± 0.3b*A*	215 ± 4*B*	33 ± 2c	457 ± 25*AB*
	'47'	3.5 ± 0.2aA	219 ± 7B	13 ± 1a	307 ± 21
rP	NAM	13.7 ± 3.0B	197 ± 11b	33 ± 10	490 ± 59b
	Pla	10.0 ± 1.4**A**	188 ± 6b	31 ± 4	524 ± 46b
	'34'	9.3 ± 1.5*A*	178 ± 7b*A*	34 ± 8	556 ± 68b*B*
	'47'	7.1 ± 0.3AB	121 ± 3aA	13 ± 1	278 ± 10a
sP	NAM	21.8 ± 0.9bC	210 ± 6b	27 ± 3	327 ± 22ab
	Pla	19.1 ± 2.0ab**B**	217 ± 6b	32 ± 3	500 ± 57b
	'34'	18.9 ± 1.2ab*B*	209 ± 16abA*B*	29 ± 1	345 ± 23ab*A*
	'47'	15.0 ± 0.9abB	148 ± 7aA	24 ± 4	306 ± 35a
P (f)		*<0.001*	*<0.001*	*0.038*	*0.005*
P (m)		*0.003*	*<0.001*	0.002	*<0.001*
P (f x m)		0.031	0.002	0.107	*0.035*

A mycorrhizal effect could be seen within the nP treatment, where shoot P content was significantly higher in treatment '34' than in treatment '47'. In the sP treatment, shoot P content was highest in the NAM treatment, though not significantly (Tab. 6). Over all P treatments, shoot K content was significantly higher in treatments NAM, 'Pla', and '34' than in treatment '47'. Shoot Zn content was significantly increased within the rP treatment by the NAM, 'Pla', and '34' treatments. Whereas within the sP treatment, the only significant difference in shoot Zn content was that treatment 'Pla' had a higher value than that of treatment '47'.

The sum of the shoot nutrient contents from both harvests of the rP treatment of experiment 2 are summarized in Tab. 7. The four mycorrhizal treatments did not differ in their total shoot P content. Treatment '47' had significantly lower total shoot K, Mg,

and Zn contents in comparison to the other three mycorrhizal treatments, the 'Pla' treatment, and the 'Pla' and '34' treatments, respectively.

Tab. 7: *Experiment 2.* Sum of shoot nutrient (P, K, Mg, and Zn) content of loose-leaf lettuce plants harvested seven and ten weeks after planting. Plants were grown on rock phosphate (rP) and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with three mycorrhizal inocula (Pla, '34', '47'). Effect of the mycorrhizal treatment (m) was tested with a one-way ANOVA. Small letters denote significant differences between means as determined by the Dunnett T3-test (P<0.05). Values are means of 4 observations ± SE.

		Total element content of two harvests			
		mg pot^{-1}			µg pot^{-1}
		P	K	Mg	Zn
rP	NAM	24 ± 5	241± 12b	52 ± 13ab	669± 69ab
	Pla	23 ± 3	253 ± 7b	72 ± 9b	882 ± 50b
	'34'	22 ± 2	230 ± 8b	67 ± 8ab	876 ± 71b
	'47'	18 ± 1	158 ± 3a	32 ± 1a	515 ± 22a
P (m)		0.488	*<0.001*	*0.028*	*0.001*

3.5 Discussion

3.5.1 Mycorrhiza colonization

The commercially available peat substrates did not contain any infectious mycorrhizal propagules, but inoculated mycorrhiza could develop in it and colonized lettuce plant roots at a sufficient rate. Mycorrhizal colonization and growth of extraradical mycelium can be suppressed by a high P supply such as that provided by fertilization with highly soluble mineral fertilizer (05b; Gryndler et al., 05a; Valentine et al., 01). It was therefore expected to have increasing root length colonization with decreasing P availability. This was verified in the first experiment. In the second experiment, root length colonization was highest on the rP treatment. Root length colonization on soils fertilized with slowly soluble rock phosphate has been described to increase compared to the colonization rate observed on soil low in phosphate (Alloush and Clark, 01), but these findings were observed on acidic soils (< pH 5), whereas the substrate used in the second experiment was only slightly acidic (pH 6.0). Perhaps the high colonization rates observed in the rock phosphate treatment of the current study are evidence that the plants sensed the large P pool and, in their eagerness to exploit it, supported mycorrhizal growth. The regulation of AM hyphal growth by plants has been clarified (Vierheilig,

04; Pinior et al., 99). The differences in root length colonization between the two experiments may be explained by the longer growth period of the second experiment.

3.5.2 Comparison of shoot dry weight and shoot elements of all treatments

Shoot N, Cu (Exp. 1) and P (Expt. 1 & 2) concentrations were low in comparison to the literature (Bergmann, 93), whereas shoot K, Zn (Exp.1 & 2) and Mg (Exp. 2) concentrations were in the medium to high range.

Shoot dry weight. Plant growth is dependent on nutrient availability to the plant. Because rock phosphate is a slowly soluble fertilizer, it was expected that the plants of the rP treatment would gain a smaller dry weight than those of the sP treatment. This was demonstrated in the first experiment, where shoot dry weight increased with solubility and amount of P fertilizer. In contrast to the first experiment, shoot dry weights in the rP and sP treatments of the second experiment were equal at both harvests, meaning that the amount of P fertilizer available to the plant had an influence on growth, but not the solubility of the different P fertilizers. Plant P availability of the slowly soluble rock phosphate must have been similar to that of the highly soluble P fertilizer.

Rock phosphate can be solubilized by a decrease in pH or by microorganisms (Villegas and Fortin, 01). The initial pH was only slightly acidic (pH 6.0) in the second experiment. Moreover, a significant positive effect on shoot dry weight by the microorganism AM fungi was not visible in both experiments. In the second experiment, highest dry weight was observed in the 'Pla' treatment at the first harvest and in treatment '34' at the second harvest, but the variance was too high to reveal significant differences compared to the NAM treatment. In treatment '47', plant dry matter production was significantly reduced on the rP treatment at the second harvest. This effect has been regularly observed (Koide and Mosse, 04; Lerat et al., 03). It has been explained as resulting from the plant's becoming independent of the fungal nutrient contribution, while continuing to loose carbohydrate needed for plant growth to the fungus.

Therefore the reasons for the higher plant availability of rock phosphate could have been several: first, after fertilization the pH might have been reduced, increasing plant-available P; second, the peat substrate or the mycorrhizal inocula might have contained bacteria that were able to solubilize rock phosphate; and third, most likely the absorptive surface area of the plants treated with rock phosphate had been increased. The investigation of the elements in the shoot gives further information for the interpretation of these results.

Shoot elements. In both experiments it was expected that AM fungi may increase P uptake into the plant, as observed on soils low in P (Grimoldi et al., 05; Asghari et al., 05). In the first experiment, AM played a role in the uptake of N and Zn only. This cor-

responds with a finding in the literature (George, 00), but the dry weight was not increased, indicating that increased N concentration was not high enough to contribute to the growth of the plant. A reason for the missing mycorrhizal P effect might be the fine root architecture of lettuce. Plants that develop a dense finely branched root system with many root hairs are less dependent on mycorrhiza (Baylis, 72). Tests with hairless mutants showed that mycorrhiza hyphae take over the function of root hairs indicating that they have similar responsibilities (Jakobsen et al., 05; Chen et al., 05). It has also been shown that AM fungi can take over the full function of P uptake by root hairs, without showing any effects in growth, P content, or AM colonization (Smith et al., 04).

In the second experiment, shoot P concentration and content on the nP treatment were increased by treatment '34' (compared to treatment '47'), but did not differ from the NAM treatment. In the rP and sP treatments, no positive mycorrhizal effect on shoot P concentration or content was found. In contrast, the NAM treatment had the highest means. This could be due to the fact that the plants were sufficiently supplied with P and the contribution by the fungus was not necessary (Koide and Mosse, 04; Lerat et al., 03), although comparison with shoot P concentrations in the literature (Bergmann, 93) suggested low P supply in the rP and sP treatments as well as in the nP treatment.

Potassium, Mg and Zn have also been reported to be increased by mycorrhiza (George, 00; Smith and Read, 97), but their shoot concentrations were not increased by any live mycorrhizal treatment over those of the NAM treatment. Comparing the three P fertilization treatments among each other, shoot Mg and Zn concentrations of treatment '34' increased in the order sP < rP < nP. This indicates that the mycorrhizal strain '34' is suitable for the contribution of Mg and Zn to plants grown on peat substrates with low plant phosphate availability.

The low P concentrations in the nP treatment in both experiments and the observed increase in shoot P concentration in the order nP < rP < sP in the second experiment, mirrored nicely the availability of the P fertilizer in the substrate. Moreover, shoot P contents revealed that the transport and accumulation of P from the substrate into the shoot was higher in the sP treatment than in the rP and nP treatment. This confirms the hypothesis that during seven weeks of growth the soluble P fertilizer was available in higher amounts than rock phosphate.

The rising shoot K and Zn concentrations in the nP and/or rP treatments compared to the sP treatment were in the first experiment a result of slower growth and thus less dilution of these elements in the plant tissue (Tab. 3 & Tab. 4). In the second experiment, at least in treatments '34' and '47', the high shoot K concentrations were not an effect resulting from slow growth only, because shoot K content was also higher in the

nP treatment than in the rP and sP treatments (Tab. 6). Although not always significant, shoot Zn content was slightly higher on the rP and nP than on the sP treatment (Tab. 6). These findings, in conjunction with the equal shoot Mg and Zn contents in the nP and rP treatments, lead to the assumption that Mg and Zn transport and accumulation into the shoot were supported by the low P availability of the substrate. Most nutrient uptake in plants is driven by the electrochemical gradient across the plasma membrane, of which a major part is induced by the H^+-ATPase activity, which is also responsible for the uptake of K, Zn, (Gilroy and Jones, 00) and probably also Mg. The main sites of H^+-ATPase activity and nutrient uptake are the root hairs, which have been shown with *Arabidopsis* to increase density extremely on P-deficient soils (Gahoonia and Nielsen, 98; Ma et al., 01). Hence one mechanism for the higher shoot content of K, Zn and Mg on the nP and also partially on the rP treatment might have been higher root hair density, resulting in an increased root surface and nutrient uptake in the NAM treatment. In the 'Pla', '34' and '47' treatments, it is possible that the AM had taken over the responsibilities of the root hairs (Jakobsen et al., 05; Chen et al., 05) leading to the same result.

Shoot N content in the rP treatment of the first experiment was an exception (Tab. 3). The rP treated plants not only had a significantly higher shoot N concentration, but also a shoot N content equal to that of the sP treatment. It may be assumed that sP treated plants grew better because of a higher uptake of phosphate, but were not able to cover their increased N demand by mobilising more N from the horn meal than the rP treated plants.

3.5.3 Comparison of mycorrhiza inocula of different origin on rock phosphate

Treatment with live mycorrhizal fungi on rock phosphate failed to significantly increase shoot dry weight, P concentration, or P content of lettuce. It can therefore be assumed that the tested AM isolates did not increase the ability of lettuce plants to mobilize rock phosphate over that of non-mycorrhizal controls. Mycorrhiza was reported to take up P from rock phosphate and increase P concentration and content in *Zea mays* L. grown on acidic soils (Alloush and Clark, 01), and in combination with pH-reducing phosphate-solubilizing rhizobacteria *Enterobacter* sp. (Barea et al., 02). Evidently, no pH-reducing or phosphate-solubilizing bacteria were present in the commercial substrates used in this study. The pH of the commercial peat substrate was probably too high to contribute to the mobilization of P from rock phosphate, although the pH was not tested at the end of the experiment. A mycorrhizal effect has been reported with hairless barley mutants, whereas their mycorrhized wild relatives (*Hordeum vulgare*) had decreased dry weight and shoot P content on rock phosphate (Chen et al.,

05). The authors suggested that the wild type's finely branched root system rendered it independent of AM-mediated P acquisition. Similarly, lettuce plants develop a very fine and dense root system and may not have to rely on mycorrhizal mycelia to provide the surface area needed for efficient P acquisition.

The only AM effect observed on rock phosphate was an increase of the shoot N content compared to the NAM treatment in the first experiment (Tab. 3). In the second experiment ‘Pla’ and ‘34’ treatments could increase Mg, K, or Zn content compared to the ‘47’ treatment only, but not compared to the non-mycorrhizal plants (Tab. 7).

It can be concluded that (a) the rate of colonization of lettuce roots by AM mycorrhizal fungi was not significantly affected by the P fertilization treatments used in this study, (b) mycorrhization increased lettuce shoot N, Mg, and Zn concentrations, but not dry weight or P concentration, (c) dry matter production of lettuce was not dependent on mycorrhizal hyphae to solubilize and take up P either from rock phosphate or from the low P treatment, probably because of the finely branched root architecture of lettuce plants, (d) slow-release P fertilization did not provide a benefit, compared to soluble P fertilization, in terms of plant dry weight over a 10-week growth period, (e) the commercial peat substrate probably did not included any pH-reducing or phosphate-solubilizing bacteria that would interact with AM, (f) when P availability was low, lettuce plants increased their shoot K, Mg, and Zn uptakes, probably by increasing the root surface area via increased root hair or external hyphae density, (g) loose-leaf lettuce was superior to cabbage lettuce in the utilization of P gained from rock phosphate, producing similar dry weight as with soluble P fertilizer, and (h) of the AM inocula tested, both, the commercially available 'Pla' inoculum and isolate '34' from an organic-dynamically managed soil performed best on the rock phosphate treatment, compared to isolate '47' from a nature conservation area. It can be speculated that isolate '47' was not efficient in using rock phosphate because natural conservation areas are generally not fertilized and therefore species with qualities others than fertilizer use efficiency have been established. Arbuscular mycorrhizae, particularly colonization by '47', tended to decrease plant ability to respond to rock phosphate fertilization, compared to the non-mycorrhizal control.

Acknowledgement: I thank Jan Dober for the contribution to this chapter with his study project and diploma thesis.

Chapter 4

4 Performance of ornamental plants grown on organic compost substrate and inoculated with arbuscular mycorrhizal fungi

4.1 Abstract

Two challenges frequently encountered in the production of ornamental plants in organic horticulture are: 1) the rate of mineralization of P and N from organic fertilizers can be too slow to meet the high nutrient demand of young plants, and 2) the exclusive use of peat as a substrate for pot-based plant culture is discouraged in organic production systems. In this situation the use of beneficial soil microorganisms in combination with high quality compost substrates can contribute to adequate plant growth and flower development. In this study, possible alternatives to highly soluble fertilizers and pure peat substrates were examined using pelargonium [*Pelargonium peltatum* L'Her.] and poinsettia [*Euphorbia pulcherrima* Willd. ex Klotzsch] as test plants. Plants were grown on peat-based substrates with different rates of compost addition (Experiment 1) and with and without arbuscular mycorrhizal (AM) fungi (Experiment 1 & 2). Inoculation with all three commercial AM inocula resulted in colonization rates of up to 36% of total root length for pelargonium and 2% for poinsettia, whereas non-inoculated plants remained free of root colonization. Increasing the rate of compost addition increased shoot dry weight and shoot nutrient concentrations, but fertilization with compost did not always completely meet plant nutrient demand. Mycorrhizal colonization increased the number of buds and flowers in both plant species as well as shoot P and K concentrations in pelargonium, but did not significantly affect shoot dry matter or shoot N concentration. It is concluded that addition of compost in combination with mycorrhizal inoculation has the capability to improve plant nutrient status and flower development.

4.2 Introduction

Two critical factors in the commercial production of flowering ornamental plants are choice of growth substrate and choice of fertilization method. Most pot-grown ornamental plants are produced and sold in peat-based substrates. In conventional production systems, these substrates are usually supplemented with soluble fertilizers in order to achieve optimal supply of nutrients such as N and P.

In organic horticulture, however, the use of synthetic chemical fertilizers is discouraged. In such horticultural systems the activity of soil microorganisms is central for the

mobilization of nutrients in the substrate (Herrmann and Plakolm, 91). Moreover, the use of peat is viewed critically, because peat is a limited natural resource, and use of peat at the present rate is not sustainable (George and Eghbal, 03; Joosten and Clarke, 02). The European Union (04) and organic growers' associations of many countries published official guidelines for organic growers to demand the use of organic and non-soluble fertilizers. They also support a reduction of peat addition to growth substrates to a maximum of 70% in the next few years (George and Eghbal, 03). These guidelines generate problems for horticulturists, because many ornamental plants require a high nutrient availability in a short growth period. In addition, low quality ornamental plants with deficiency symptoms can not be marketed.

To meet the challenge for a successful economic ecological greenhouse horticulture on a long-term it is important (a) to characterize methods to improve nutrient supply from organic sources, and (b) to find alternative substrates for peat in pot cultures without loss of plant quality.

To reduce peat at least partly in growth substrates, various alternative organic materials, such as bark, coconut residues (Linderman and Davis, 03c; Ozores-Hampton et al., 99), or compost (Veeken et al., 04), have been tested. Compost is a substrate that is traditionally used in agriculture and horticulture due to its beneficial effect, for example, on soil stability or soil biota (Carpenter-Boggs et al., 00; Wells et al., 00). However, in many modern greenhouse horticultural systems producers avoid compost application, because they fear the transmission of plant diseases. This risk is relatively low in high quality compost. It can not only be almost free of pathogenic microorganisms, but may even benefit the plants by suppressing soil born diseases (Schüler et al., 89) and providing a high nutrient content. Therefore at least 20% high quality compost is recommended to be mixed with peat for a substrate currently used in organic horticulture in Germany and Switzerland (König, 04; Bioland, 05; Naturland, 06; Demeter, 04).

The sole application of compost as nutrient source for plants may require additional amendment of other organic fertilizers to meet the plant demands. Moreover, soil microorganisms may help plants to improve the plant nutrient status. A group of soil microorganisms living in symbiosis with several plant species are the arbuscular mycorrhizal (AM) fungi. These fungi are capable to support the uptake of nutrients into the plant, such as phosphorus (P), nitrogen (N), zinc (Zn), copper (Cu), and sometimes potassium (K) (George, 00), and to increase plant dry weight on soils low in these nutrients (Douds et al., 05). For AM-colonized plants P from organic fertilizers may be particularly accessible (Linderman and Davis, 04). In addition, AM colonization of plants resulted in suppression of diseases (Kasiamdari et al., 02), and more resistant to stresses such as drought (Neumann and George, 04; Pinior et al., 05) or salinity (Tian et al., 04). In soils AM fungi can also stabilize soil aggregates (Piotrowski et al., 04).

Plant and fungus exchange several signals in the roots, including hormones like the cytokinin isopentenyl adenosine, the auxin conjugate indole-3-butyric acid, or gibberellins (Barker and Tagu, 00; Shaul-Keinan et al., 02; Fitze et al., 05).

Arbuscular mycorrhizal colonization may induce earlier flowering and increased flower numbers (Nowak, 04; Gaur and Adholeya, 05; Usha et al., 05). This trait of AM fungi is of particular interest to horticultural production. Floral development starts after a period of vegetative growth, during which the plant maximizes leaf area for high photosynthesis (Krizek and Fletcher, 05). The major signals for flower development are e.g. photoperiod, gibberellin, and vernalization, as tested in *Arabidopsis thaliana* mutants (Jack, 04). Pelargonium is independent of day length and starts flowering after a certain number of light hours, whereas flowering of poinsettia is induced by a short day photoperiod (Elsner et al., 95; Zimmer, 91).

Variation in the levels of P and K supplied to plants has been shown to affect flowering in some instances. As an example, a higher P supply is recommended for optimal development of poinsettia flowers compared to the earlier vegetative growing period (Zimmer, 91). Poulton et al. (02) observed a significantly higher number of tomato flowers and slightly increased shoot P concentration with mycorrhizal colonization on low P substrates, whereas shoot dry weight was not significantly affected. On peat substrate with organic NPK fertilizer, mycorrhizal pelargonium plants flowered earlier and had increased N, P, and K concentrations at low nutrient supply as well as increased P concentrations at high nutrient supply, while the number of flowers and the leaf dry weight were unaffected (Nowak, 04). *Zinnia* and *Tagetes* plants had an increased number of flowers after mycorrhization, but final dry weight as well as K and P concentrations were unaffected (Aboul-Nasr, 96). Gaur and Adholeya (05) tested five ornamental plant species on a soil:compost mixture and found earlier flowering, a significantly higher number of flowers, and increased shoot P concentrations only for *Callistephus*. The flower timing of cotton was not changed by different K supply and leaf K concentrations (Reddy and Zhao, 05).

The effect of compost addition on mycorrhizal and non-mycorrhizal plant seedlings has been only scarcely investigated. Compost containing substrates may be appropriate for mycorrhizal plants (Goswani and Jamaluddin, 01; Linderman and Davis, 01) if the quality of the compost is adequate (Boddington and Dodd, 00; Raviv M et al., 98; Perner et al., 06). Anyway, compost amendment may suppress mycorrhizal colonization and therefore the activity of AM fungi (Sáinz et al., 98). Thus, until now it is not clear whether mycorrhizal inoculation in combination with compost addition increase yield and flower production in organic management systems.

Therefore, we utilized poinsettia and pelargonium as test plants in two experiments studying whether (a) increasing the rate of compost application contributes to plant dry

weight and N, P, and K supply, (b) commercially- or specifically-prepared peat-compost substrates support AM fungus colonization of plants, (c) AM fungus colonization is beneficial to plants on these substrates with regard to dry weight and N, P, K, and Zn supply, and (d) AM fungus colonization increases production of flowers and buds. The aim was to increase the understanding of the role of AM fungi in plant growth on organic substrates.

4.3 Material and Methods

4.3.1 Overview of experimental design and cultivation

In Experiment 1, single seedlings of pelargonium (*Pelargonium peltatum* 'Balcon Imperial Compact', Silze, Weener Halte, Germany) were placed in separate 250-ml pots filled with a peat substrate with an addition of 20% or 40% compost. Drip irrigation (40 $ml \cdot min^{-1}$) was used every second day (total of 40 ml) to maintain favorable water conditions in the substrate. Additionally, every 3rd or 4th day the pots were weighed to equalize the water content of the pots. The experiment was carried out from 11 Sep. to 23 Oct. 2002 (6 weeks) in a greenhouse facility at Großbeeren (long. 13°19´60´E; lat. 51°22´0´N), Germany. Average air temperature in the greenhouse during this time was 23 °C (min 17 °C and max. 27 °C) during the day and 18 °C (min. 17 °C and max. 25°C) at night. Relative humidity was on average 66% during the day and 77% at night. The daily (10.5 h) mean light intensity (PAR) was 8 $mol \cdot m^{-2}$ (max. 662 $\mu mol \cdot m^{-2} \cdot s^{-1}$).

In Experiment 2, single seedlings of poinsettia (*Euphorbia pulcherrima* 'Cortez Red', IGZ, Erfurt) were placed in separate 500 ml pots filled with a commercial growth substrate (see below). The plants were grown from 27 March to 22 May 2003 (8 weeks) in the greenhouse facilities at Großbeeren. The average air temperature in the greenhouse during this time was 24 °C (min. 18°C and max. 32°C) during the day and 20 °C (min. 16 °C and max. 24 °C) at night. Relative humidity was on average during the day 77% and at night 82%. The daily (12.5 h) mean light intensity (PAR) was at 13 $mol \cdot m^{-2}$ (max. 1337 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during the day. Pots were rearranged at regular intervals in both experiments. Pots were always arranged in a completely randomized design in both experiments.

4.3.2 Substrate preparation and characterization

All substrates used in this study were suitable for organic production. The compost was prepared from yard waste, shredded trees and bushes (Bruns, 98; Bruns and

Schüler, 00). The material used had a wide C/N ratio (40:1) at the beginning of the composting process. After three months of composting the extractable nutrient content in the compost of Experiment 1 (pelargonium) was: N, 150 mg·L^{-1}; P, 360 mg·L^{-1} and K, 1535 mg·L^{-1} (extraction by $CaCl_2$ [N] and CAL [P, K]; C. Bruns, personal communication). The compost had a salt content of 2.2 g·L^{-1} and a pH ($CaCl_2$) of 7.1. The compost was mixed with sphagnum peat from the Baltic region (white peat) to obtain a compost substrate with 20% or 40% compost by volume. The substrates were limed with CaO to a pH of 6.2 and sieved to 10 mm. In addition, N fertilizer was added to the substrate one day before the start of the experiment. The N fertilizer (a mixture of 33% horn meal 0-2 mm, containing 10% N, and 66% horn meal 2-6 mm, containing 14% N) was uniformly mixed into the substrate (20%: 6700 mg·L^{-1} and 40%: 5500 mg·L^{-1}).

In previous work with this substrate and fertilizer (C. Bruns, personal communication), it had been observed that 25% of the added N became available to plants within two weeks after planting, and that within eight weeks after planting 85% of the added N was available. Therefore, the plant-available N content of the compost substrate together with the horn meal fertilizer added by calculation up to 200 mg·L^{-1} in the first two weeks after planting in both compost addition treatments.

In experiment 2 with poinsettia plants, a commercial substrate (KKS Bio-Potgrond, Klasmann-Deilmann GmbH, Geeste-Gross Hesepe, Germany) was used that contained approximately 80% (v/v) sphagnum peat (black peat) and approximately 20% (v/v) compost of green residues. The substrate also contained clay material, lime, horn meal, and Thomas phosphate. This substrate is commonly used by organic growers in Germany. The extractable nutrients (extraction by $CaCl_2$ [N] and CAL [P, K]; information from the supplier) in this substrate were: N, 300-400 mg·L^{-1}; P, 109-153 mg·L^{-1}; and K, 290-415 mg·L^{-1}. The substrate had a salt content of 1-2 g·L^{-1} and a pH ($CaCl_2$) of 5-6.

4.3.3 Inoculation with AM fungi

Inoculation with AM fungi in both experiments was carried out with three different commercially available inocula: Pla (TerraVital Hortimix with *G. mosseae, G. intraradices, G. claroideum* and *G. microaggregatum*, >50 infective units per ml inoculum; Plantworks Ltd., Heeley Close, Sittingbourne, Kent, UK), Bio (Endorize-Mix with *G. mosseae, G. intraradices, Glomus sp.*, infective units not specified; Biorize, Rue Sainte Anne, Dijon, France), and Tri (*G. mosseae*, *Glomus intraradices,* and *G. etunicatum*, 50 infective units per ml inoculum; Triton, AMykor GmbH, Wolfen, Germany). The inocula were mixed uniformly into the potting substrate before planting the seedlings. Addition rates were used according to the suppliers' recommendation and were: Pla, 5% (v/v); Bio, 5% (v/v); and Tri, 3% (v/v). Non-mycorrhizal (NAM) treatments were

supplied with autoclaved (121°C for 20 min) Pla inoculum. In addition, a filtrate (589/3 blue ribbon paper filter, Schleicher & Schuell Bioscience GmbH, Dassel, Germany) of non-sterilized Pla inoculum was added to NAM pots in an effort to supply similar amounts of nutrients and microorganisms other than AM fungi to all treatments.

4.3.4 Harvest and plant analysis

Pelargonium buds and flowers were counted and removed three times during the experiment, and the individual counts were combined. Poinsettia buds and flowers were counted at the end of the experiment. Shoots were separated from roots, and shoot fresh weight (FW) was recorded. Shoots were then dried at 80°C for two days, and dry weight (DW) was recorded. The shoots were ground in a centrifugal grinder using a 0.25-mm sieve. Shoot samples were dry ashed and dissolved in 18.5% HCl. Potassium and Zn were analyzed with an atomic absorption spectrophotometer (Perkin Elmer 3300, Überlingen, Germany). Phosphorus was analyzed photometrically with an EPOS-Analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar Vario EL, Hanau, Germany).

For the investigation of root dry weight the whole pot (Expt. 1) or one quarter of the pot (Expt. 2) was washed to separate the roots from the substrate with running cold water using a set of sieves (smallest sieve size 1 mm). The root FW and DW were recorded and a representative subsample for assessment of mycorrhizal fungus colonization was taken and stored in 10% isopropanol. Mycorrhizal fungus colonization of roots was determined following the method of Koske and Gemma (89) with slight modifications. Roots were cleared with 10% KOH, acidified with 2 N HCl, and stained with 0.05% trypan blue in lactic acid. The colonization rate of poinsettia roots is difficult to determine under the microscope, because poinsettia roots are very hard and darkened quickly. A reason could be the latex in the cell sap of poinsettia plants (Ibanez et al., 04). Therefore, poinsettia roots were additionally bleached with 2% H_2O_2 solution. Percentage root length colonization was determined with a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 100x using the grid line intersection method (Giovannetti and Mosse, 80).

4.3.5 Statistics

In Experiment 1, data were analyzed by a two-factorial analysis of variance, with compost addition rate and mycorrhizal inoculation as experimental factors (n = 4). Data in Experiment 2 were subjected to a one-way analysis of variance, with inoculum type as experimental factor (n = 4). Mean separation was carried out with the Tukey-test ($p < 0.05$) on case of variance homogeneity, otherwise with a Wilcoxon-test ($p <$

0.05). Individual treatment differences were subject to t-test. Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, U.S.) software.

4.4 Results

4.4.1 Experiment 1

Colonization of pelargonium roots by AM was not significantly different between the two compost addition levels. Average root length colonization across both compost addition levels for the different inocula was (± SE): Pla, 36 ± 4%; Bio, 34 ± 4%; and Tri, 15 ± 5%. Non-inoculated plants remained free of mycorrhizal colonization, although the substrate has not been sterilized before use.

Tab. 8: *Experiment 1* (pelargonium). Shoot and root dry weight (DW), and number of buds and flowers of pelargonium plants six weeks after planting. Plants were grown on compost-peat substrate with 20% compost or 40% compost addition rate, and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with one of three mycorrhizal inocula (Plantworks (Pla), Biorize (Bio), Triton (Tri)). Effects of the treatments (mycorrhizal inoculation (m); compost addition rate (c)) were tested with a two-way ANOVA. Different letters denote significant differences between means within one factor as determined by the Tukey test (P<0.05). Values are means of 4 observations ± standard error of the mean (SE).

	Shoot DW g pot^{-1}	Root DW g pot^{-1}	Buds & flowers n° pot^{-1}
inoculum			
NAM	2.4 ± 0.1	0.19 ± 0.02a	9 ± 3a
Pla	2.3 ± 0.1	0.16 ± 0.03a	18 ± 3b
Bio	2.5 ± 0.1	0.20 ± 0.04ab	16 ± 2ab
Tri	2.5 ± 0.1	0.27 ± 0.02b	15 ± 4ab
compost			
20%	2.3 ± 0.1a	0.21 ± 0.02	13 ± 3
40%	2.5 ± 0.1b	0.20 ± 0.03	15 ± 2
P (m)	0.362	*0.009*	*0.039*
P (c)	*0.003*	0.444	0.387
P (m x c)	0.845	0.087	0.967

Shoot dry weight was significantly higher on 40% compost than on 20% compost substrate, but it was not significantly affected by inoculation with AM (Tab. 8). Root dry weight was not influenced by compost treatment, but was significantly enhanced in the Tri treatment compared to the NAM treatment (Tab. 8).

The number of buds and flowers was not significantly influenced by compost addition rate (Tab. 8). The Pla treatment significantly increased the number of buds and flowers compared to the NAM treatment. Comparison of the individual treatment combinations (Fig. 7) showed that on the 40% compost substrate, the number of buds and flowers was higher also in the Bio and Tri treatments than in the NAM treatment.

Tab. 9: *Experiment 1* (pelargonium). Concentration and content of N, P, and K in shoots of pelargonium plants six weeks after planting. Plants were grown on compost-peat substrate with 20% compost or 40% compost, and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with one of three mycorrhizal inocula (Plantworks (Pla), Biorize (Bio), Triton (Tri)). Effects of the treatments (mycorrhizal inoculation (m); compost addition rate (c)) were tested with a two-way ANOVA. Different letters denote significant differences between means within one factor determined by the Tukey test (P<0.05). Values are means of 4 observations ± SE.

	Element concentration g·[kg DW]$^{-1}$			Element content mg·pot^{-1}		
	N	P	K	N	P	K
inoculum						
NAM	26.1 ± 0.8	2.1 ± 0.0a	9.2 ± 0.7a	62.1 ± 2.3	5.0 ± 0.2a	21.7 ± 1.3a
Pla	26.4 ± 0.4	2.6 ± 0.1b	21.0 ± 1.6c	60.1 ± 2.9	5.9 ± 0.2b	47.4 ± 2.7c
Bio	25.8 ± 0.9	2.7 ± 0.1b	14.9 ± 1.0b	63.7 ± 2.1	6.8 ± 0.2c	36.5 ± 1.5b
Tri	24.3 ± 1.7	2.2 ± 0.1a	9.9 ± 0.9a	59.8 ± 2.5	5.5 ± 0.2ab	24.4 ± 2.4a
compost						
20%	25.1 ± 0.8	2.0 ± 0.1a	14.2 ± 1.0	56.7 ± 1.8a	4.5 ± 0.2a	31.9 ± 2.5
40%	26.2 ± 1.1	2.8 ± 0.1b	13.3 ± 1.1	66.1 ± 3.2b	7.1 ± 0.3b	33.1 ± 1.5
P (m)	0.281	*<0.001*	*<0.001*	0.448	*<0.001*	*<0.001*
P (c)	0.176	*<0.001*	0.259	*<0.001*	*<0.001*	0.476
P (c x m)	0.311	0.923	0.896	0.769	0.575	0.301

Shoot N and K concentrations were not significantly different between the 20% and 40% compost substrates (Tab. 9). Shoot P concentration was significantly higher on 40% than on 20% compost substrate. Inoculation did not induce significant differences in shoot N concentration (Tab. 9). Shoot P and K concentrations were significantly increased by the Pla and Bio treatments on both substrates. The concentration of K in shoots was especially high in the Pla treatment. The shoot Zn concentration was not influenced by AM or compost addition rate (data not shown).

The 40% compost treatment significantly increased shoot content of N and P compared to the 20% compost substrate (Tab. 9), whereas shoot K content was not influenced by compost additional rate. Inoculation with AM did not induce significant dif-

ferences in shoot N content. Compared to the NAM treatment, shoot P and K content were significantly increased by the Pla and Bio treatments, with P content highest in the Bio treatment and K content highest in the Pla treatment.

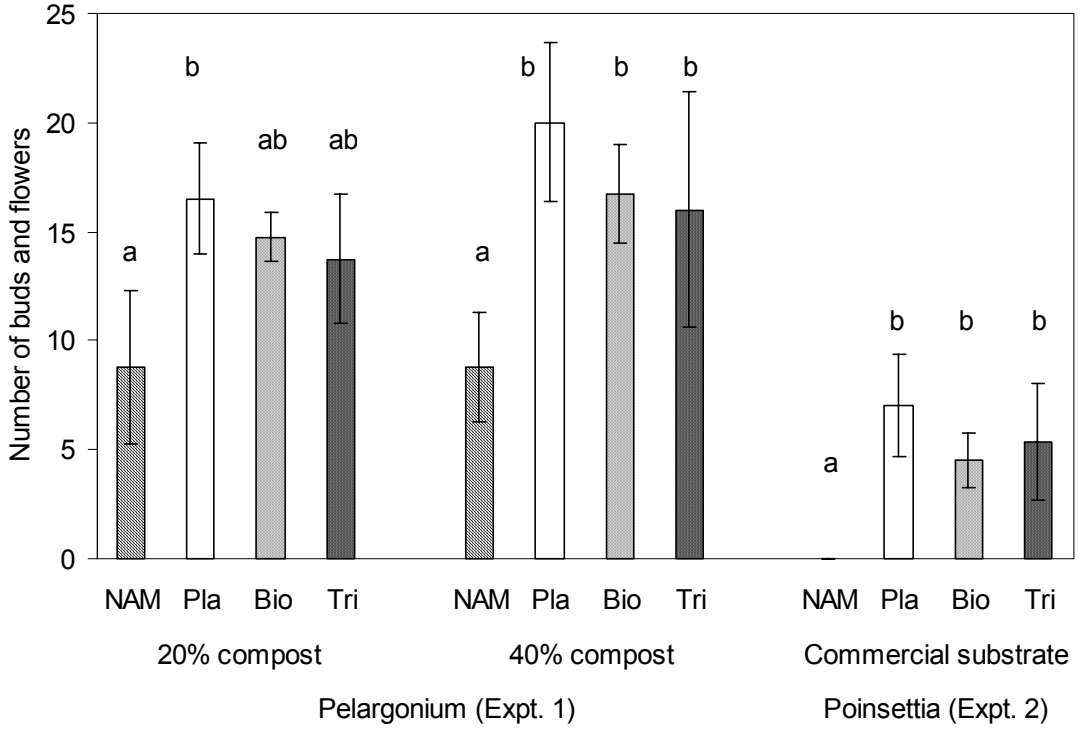


Fig. 7: Number of buds and flowers of pelargonium and poinsettia plants six (Exp. 1) and eight (Exp. 2) weeks after planting in compost-peat substrates (*Exp. 1*), or on commercial growing substrate (*Exp.2*). In both experiments, plants were either non-inoculated with mycorrhizal fungi or were inoculated with one of three mycorrhizal inocula (Plantworks (Pla), Biorize (Bio), Triton (Tri)). Different letters denote significant differences between means of mycorrhizal inoculation treatmenrs determiened by a Tukey test (Exp. 1; $P<0.05$) or by a Wilcoxon test (Exp. 2; ($P<0.05$). Means of 4 observations ± SE (T).

4.4.2 Experiment 2

Mean root length colonization of poinsettia plants on the commercial substrate for all three inocula was 2±1%. Although many roots were rated as non-mycorrhizal, mycorrhizal structures were well expressed in some part of the root system. The non-inoculated plants remained free from mycorrhizal colonization. Shoot dry weight was not significantly affected by inoculation, although the NAM treatment had the highest and the Tri treatment the lowest dry weight (Tab. 10). Root dry weight was also not significantly affected by inoculation.

Tab. 10: *Experiment 2* (poinsettia). Shoot and root dry weight, and shoot element (N, P, K) concentrations, and shoot N content of poinsettia plants eight weeks after planting. Plants were grown on a commercial compost substrate, and were either non-inoculated with mycorrhizal fungi (NAM) or were inoculated with one of three mycorrhizal inocula (Plantworks (Pla), Biorize (Bio), Triton (Tri)). Effects of the treatment (mycorrhizal inoculation (m)) were tested with a one-way ANOVA. Values are means of 4 observations ± SE.

	Dry weight		Element concentration		
	g pot^{-1}		g [kg DW]$^{-1}$		
	shoot	root	N	P	K
NAM	9.2 ± 0.3	0.19 ± 0.04	15.5 ± 0.5	2.3 ± 0.1	14.6 ± 0.4
Pla	7.0 ± 1.1	0.18 ± 0.05	17.7 ± 2.0	2.8 ± 0.4	17.7 ± 1.6
Bio	7.6 ± 0.4	0.27 ± 0.03	15.0 ± 1.0	2.5 ± 0.2	18.5 ± 2.1
Tri	6.2 ± 1.2	0.14 ± 0.03	17.2 ± 1.7	3.2 ± 0.3	20.0 ± 1.6
P (m)	0.222	0.176	0.521	0.147	0.232

However flower and bud production was significantly increased by mycorrhizal colonization with all three types of inoculum (Fig. 7, $p < 0.05$; Wilcoxon test).

Shoot N, P and K concentrations were not significantly influenced by inoculation (ANOVA; Tab. 10). Shoot concentrations of P and K were non-significantly increased with inoculation. Comparison of individual treatments by t-test showed a significant increase of shoot P and K concentrations in the Tri treatment when compared to the NAM treatment (P: P=0.035; K: P=0.047). Shoot Zn concentration and shoot N, P and K content were not significantly influenced by inoculation (data not shown).

4.5 Discussion

Roots of inoculated pelargonium plants were well colonized with mycorrhizal fungi. This was true at both compost addition rates (20% and 40%) and for all three commercial inocula. Poinsettia, grown on a commercial substrate, in contrast had a very low colonization rate. The lower infection rate of poinsettia may not be species specific, but may rather be due to substrate properties. Further experiments on compost substrate showed that a colonization rate up to 40% is possible in poinsettia on these substrates (Perner, data unpublished). The growth of AM fungi can be suppressed in moist soils (Smith and Read, 97) or in soils with a high nutrient supply where plants are not dependent on the symbiosis (Koide and Mosse, 04).

The observation that all three horticultural substrates used in this study did not support spontaneous mycorrhizal colonization is of high practical significance. Horticul-

tural producers must use inoculation and relatively low nutrient addition rates if they intend to grow mycorrhizal plants on these substrates.

A comparison of the nutrient concentrations of the pelargonium shoots in the present study with literature values for adequately fertilized plants (Bergmann, 93) showed a sufficient supply of N and Zn to the plant in this study. At the both rates of compost addition, shoot P and K concentrations were low in the NAM and Tri treatments, whereas the plants treated with Pla and Bio were provided with higher P concentrations and adequate K concentrations. Pelargonium plants grown with the high rate of compost addition and Pla or Bio had a shoot P concentration indicating sufficiency. Increasing the rate of compost amendment of the substrate from 20% to 40% had a supporting effect on the growth of pelargonium plants. This effect may be due to increased P supply, as evidenced by the increased shoot P concentration of plants grown in the 40% compost treatment. Another reason could have been the higher water holding capacity of peat-based substrates with higher compost addition rate (Perner et al., 06).

Inoculation with mycorrhizal fungi did not result in an increased pelargonium shoot dry weight. On soils deficient in P, mycorrhizal colonization supports plant development by supplying the plant with additional P, and sometimes with N, K or Zn (George, 00; Nowak, 04). Although we found low P concentrations in shoot tissue, substrate P availability may still have been too high to allow an AM-dependent shoot enhancement effect. Alternatively, on organic substrates some mycorrhizal fungi may be less effective in P uptake than on mineral soils (Perner et al., 06).

Both a reduction and an increase of root growth upon mycorrhizal colonization has been observed under favourable conditions (Liu et al., 04; Martin and Stutz, 04). In the present experiment, the higher root dry weight in the Tri treatment had no consequences for shoot dry weight or for the number of bud and flowers of pelargonium plants.

The contribution of AM fungi to plant nutrient uptake is often particularly evident in plants that are deficient in a certain nutrient. Thus, it is not surprising that no mycorrhizal effect on pelargonium shoot N or Zn concentrations was found in the present study. In the case of pelargonium shoot P and K concentrations, a mycorrhizal effect was evident. These findings correspond with those of Nowak (04) in a study of pelargonium provided with low NPK supply. Mycorrhizal fungi are well known for their efficient P uptake, but the contribution of K to plants by AM has been described more rarely and specifically on acidic soils (see e.g., Clark and Zeto, 00; Alloush and Clark, 01). It is possible that small aggregates of compost and peat remained acidic in the limed substrate, and that hyphae entered these acidic aggregates and exploited addi-

tional K sources. Moreover, the decomposition of the organic material released humic acids with the consequence of decreased pH and increased availability.

The pelargonium shoot P concentration was not increased when Tri inoculum was used. With this inoculum AM-acquired P was likely incorporated preferentially in the roots, leading to a considerable increase in root dry weight in this treatment.

A comparison of the nutrient concentrations of the poinsettia shoots with literature values (Bergmann, 93; Zimmer, 91) indicated for the present study a sufficient supply of Zn but an insufficient supply of N. Shoot P and K concentrations were low in the NAM treatment, whereas plants treated with Pla, Bio, and Tri had concentrations of these two elements that were close to the lower limit of sufficient supply (3 g kg^{-1} for P and 15 g kg^{-1} for K; Bergmann, 93; Zimmer, 91). The low N concentrations observed in the poinsettia plants in the present study indicate that the mineralization rate of N in the substrate was inadequate to meet plant N demands.

For poinsettia, neither root dry weight nor shoot dry weight was significantly affected by mycorrhizal colonization. The observed tendency of a lower dry weight in the inoculated treatments could be a result of carbon demand of the mycorrhizal fungus that could not be compensated by the nutrients supplied by the fungus (Mortimer et al., 05).

Shoot nutrient concentrations of poinsettia were not significantly affected by AM inoculation. This may be due to the low mycorrhizal colonization rates in poinsettia or due to higher nutrient availability in the substrate used for Experiment 2. The significance of the K and P concentrations between the Tri and NAM treatments is probably due to the slower growth of the inoculated plants, which can result in elevated concentrations of P and K.

Bud

The number of buds and flowers increased with AM inoculation. A similar increase in buds and flowers of *Tagetes, Zinnia, Callistephus* and tomato with mycorrhizal colonization has been described previously (Aboul-Nasr, 96; Gaur and Adholeya, 05; Poulton et al., 02). The rate of compost amendment to the substrate had no influence on the number of buds and flowers in pelargonium, and, in both species, the number of buds and flowers did not correspond with either shoot N or shoot Zn concentration. In pelargonium, shoot concentrations of P and K were increased in the Pla and Bio treatments, but only shoot K concentration corresponded with the number of buds and flowers.

Bud and flower production of poinsettia was strongly influenced by inoculation with mycorrhiza. Shoot P and K concentrations indicated sufficiency only in the inoculated treatments. However, the effect of mycorrhizal colonization on flowering can not be

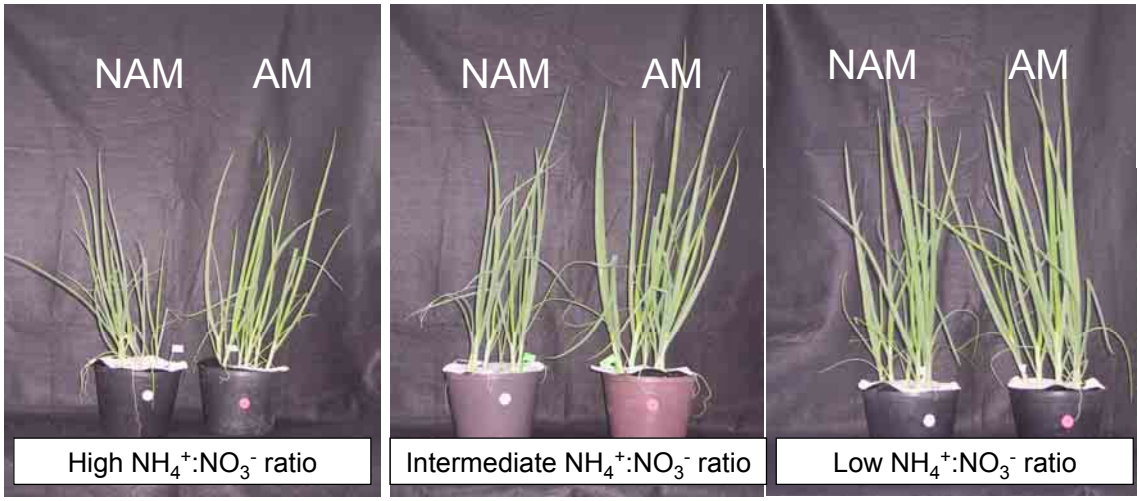
clearly linked to better nutrient status in mycorrhizal plants, because the differences in shoot nutrient concentrations between mycorrhizal and non-mycorrhizal plants were not sufficiently distinct.

Potassium is involved in a wide range of functions in plants: photosynthesis, enzyme activation, protein synthesis, osmotic potential, and as a counterion to inorganic ions and organic biopolymers (Marschner, 95). It has also been shown that K acts as a carrier ion in xylem and phloem, transporting solutes, assimilates, and hormonal stress signals such as abscisic acid (Peuke et al., 02) Higher levels of K in the plants could induce faster rates of transport of hormones, such as gibberellins, that induce bud production. Thus, mycorrhizal colonization may either directly influence plant hormoneal balance, or may indirectly affect plant hormone levels by altered plant nutrient status.

It was concluded that (a) AM colonization was established in pelargonium plants on horticultural substrates, irrespectively of varied rate of compost addition to the substrate, (b) increasing the rate of compost amendment moderately increased pelargonium shoot dry weight due to higher nutrient supply, but compost-peat substrates may still require additions of, for example, NPK sources to result in plant nutrient sufficiency, and (c) AM had no effect on shoot dry weight or shoot N concentration of the studied species, but it increased shoot P and K concentrations on compost-peat substrates low in P and K supply.

It was also conclude that bud and flower production (d) was not affected by the rate of compost amendment of the substrate, and (e) can be increased or accelerated by inoculation with a commercial mycorrhizal inoculum. Increase of bud and flower production may have been the result of AM-mediated increases in plant nutrient (especially K) concentrations in combination with a possible hormonal effect induced by the presence of mycorrhizal colonization. Mycorrhizal plants may accumulate nutrients in a shorter time span, so that they are earlier in life sufficiently supplied with nutrients to initiate flower development.

Chapter 5


NAM
AM
High NH_4^+:NO_3^- ratio
NAM
AM
Intermediate NH_4^+:NO_3^- ratio
NAM
AM
Low NH_4^+:NO_3^- ratio

5 Influence of nitrogen speciation and mycorrhizal colonization on growth and composition of Chinese bunching onion

5.1 Abstract

In recent years, interest in cultivating *Allium* species with enhanced health benefits and/or destinct flavor has grown. Concentrations of the phytochemicals determining these desired characteristics may be influenced by plant supply with sulfur and nitrogen. These relations were examined with the test plant bunching onion [*Allium fistulosum* L.], using three different ammonium:nitrate ratios in combination with an arbuscular mycorrhizal (AM) fungus (*Glomus mosseae*), in terms of changes in dry weight, nutrient composition (N, NO_3^-, P, S), singular sugars (glucose, fructose and sucrose), total soluble solids, and organosulfur compounds (measured as pyruvic acid). The experiment was carried out in a greenhouse using perlite as substrate with application of nutrient solution at regular intervals.

In non-mycorrhizal plants, low and intermediate ammonium:nitrate ratios supported adequate growth of *A. fistulosum* compared to decreased growth and wilting symptoms observed at an high ammonium:nitrate ratio. Mycorrhizal colonization drastically increased dry matter production of plants in the high and intermediate ammonium:nitrate ratio treatments. In these treatments, drainage pH was distinctly lower than at the low ammonium:nitrate ratio. Shoot N concentration was increased at the high ammonium:nitrate ratio. Shoot P concentration also increased with higher ammonium supply rate. N speciation and AM colonization had little effect on shoot sulfur concentration, sugars or soluble solid compounds. The total yield of pyruvic acid was significantly affected both by N speciation and by AM colonization. The highest pyruvic acid yield was obtained in mycorrhizal plants supplied with an low ammonium:nitrate rate. However, *A. fistulosum* plants supplied with an intermediate ammonium:nitrate ratio, also produced satisfactory pyruvic acid yield when they were mycorrhizal.

5.2 Introduction

In the following two chapters 4 and 5 two different *Allium* species were used to observe the influence of AM fungi in combination with different ammonium:nitrate ratios in N supply on the secondary plant metabolism.

Allium species are grown worldwide and are popular in many countries. They are of importance in many diets, because of their nutritional significance (Keiss et al., 03;

Bergès et al., 04). Bunching onion is a particularly popular vegetable in many regions of China. There is a growing interest in cultivation of foods with increased levels of phytochemicals that provide health benefits and have defined flavor properties (Griffiths et al., 02).

The precursors of certain of these desirable phytochemicals in *Allium* species are S-alk(en)yl-L-cysteine sulfoxides, which are biosynthetically derived from cysteine and glutathione. When tissue is damaged, these precursors are activated by the enzyme alliinase to produce pyruvic acid, ammonia and sulfenic acids. The unstable sulfenic acids react to form a variety of sulfides, thiosulfinates and minor sulfur (S) containing products (Jones et al., 04). Many of these S containing compounds and elementary S have been linked to the defense against microbial pathogens (Cooper and Williams, 04; Bloem et al., 05; Jones et al., 04; Rausch and Wachter, 05). Earlier studies have shown that the level of pyruvic acid in onion juice correlates with flavor compound pungency (Schwimmer and Weston, 61). The formation of pungent compounds and sugars (Randle, 92b; Randle and Bussard, 93; McCallum et al., 05) and especially health related organosulfur compounds (Keiss et al., 03), may be increased in *Allium* plants by an increased supply of sulfur. Other studies have not only shown that S influences organosulfur compounds, but a specific experiment conducted by Coolong and Randle (03) found that even in soils with sufficient S supply, the N level can affect the formation of organosulfur compounds, particularly the S-alk(en)yl-L-cysteine-sulfoxides. The reason is that the S uptake by roots in most higher plants is supported by high-affinity proton/sulfate transporters (Smith et al., 95; 97; Takahashi et al., 97; Hawkesford, 03). The basic regulation by S is additionally controlled by the carbon (C) and N status (Maruyama-Nakashita et al., 04; Hesse et al., 04). It was found that S uptake and assimilation is dependent as well upon the constant supply of the precursor of cysteine, o-acetylserine (OAS), which depends upon N and C availability (Koprivova et al., 00; Kopriva et al., 02). O-acetylserine holds an extraordinary position in the association of N with the S metabolism (Leustek et al., 00; Saito, 00).

The uptake of nitrate (NO_3^-) and sulfate (SO_4^{2-}) is followed by an immediate reduction to ammonia (NH_3), which is incorporated into amino acids, and sulfide (S^{2-}), respectively. Both reactions need six electrons at a time for each anion (Lüttge et al., 99). The uptake of NH_4^+ and integration into amino acid is less energy consuming than starting from NO_3^-. This could certainly be an advantage for the plant, because as a consequence it is left with higher SO_4^{2-} reduction rate which may lead to more secondary metabolites. This is particularly true for plants that are grown under insufficient light conditions to provide the plant with enough reduction equivalents.

Uptake of SO_4^{2-} is in unspecific competition with NO_3^- and has been reported to increase with increasing NH_4^+ supply (Van den Berg et al., 05). It was therefore hypothe-

sized that with a higher ammonium:nitrate ratio in supply, S uptake and subsequently organosulfur compounds will increase.

Allium plants are often colonized by AM fungi. *Allium* growth can be highly dependent on mycorrhizal colonization (Smith and Read, 97). Earlier work has shown that yield and quality of *Allium* plants may be increased after mycorrhizal colonization (Fusconi et al., 05), but results were not always reproducible. In particular, mycorrhizal effects on plant growth often occur on low-phosphorus (P) soils only. The effect is based on the characteristic of hyphae to exploit a larger soil volume than roots alone and to supply considerable amounts of P to the plant according to the plant's demand. Phosphorus is often fixed to soil particles and P exchange with the soil solution and subsequent diffusion in the soil solution slow are processes. Because of the shorter diffusion distance to the surface of hyphae than to the surface of roots, mycorrhizal plants absorb the soil P more efficiently than non-mycorrhizal plants (George, 00).

High P concentration in roots may lead to lower mycorrhizal colonization rates and to decreased growth of extraradical mycorrhizal mycelium (Sanders, 75; Bruce et al., 94; Valentine et al., 01). Therefore, it is often argued that consequences of mycorrhizal colonization are neglible in practical agriculture or horticulture where substrate P supply is often quite high. In the present experiment, plants were supplied with sufficient P in a flow through potting system. Consequently, it was expected that mycorrhizal effects on plant P uptake are minimal under the present experimental conditions.

The supporting effect of AM colonization is not only known for nutrients uptake, but it can also influence the secondary metabolism of a plant, monitored for example as earlier flowering or reduced flower ethylene production (Backhaus, 83; Besmer and Koide, 99). Mycorrhizal fungi is also beneficial for plants that are stressed by drought (Neumann and George, 04), salinity (Tian et al., 04), or heavy metals (Andrade et al., 04; Leung et al., 06; Rivera-Becerril et al., 05). Ammonium supply in high concentrations can also be toxic to plants. Plants preferentially take up NO_3^-, whereas for microorganisms NH_4^+ is the most important source of mineral nitrogen (Stitt et al., 02). Therefore we hypothesize that mycorrhiza supports the plants in their uptake of NH_4^+ and influences the secondary metabolites, such as organosulfur compounds.

Thus, the objective of the present research was to determine comprehensively the effects of (a) different supplied ammonium:nitrate ratios, and (b) mycorrhizal colonization on shoot dry weight, nutrient status (N, NO_3^-, P, S), plant composition (soluble solid compounds, glucose, fructose, and sucrose) and concentration and total content of health-related organosulfur compounds (measured indirectly as pyruvic acid) of bunching onion.

5.3 Material and Methods

5.3.1 Overview on experimental design and cultivation

Seeds of bunching onion, *Allium fistulosum* (Chinese cultivar Zhang Qui), were suspended in water with 10% H_2O_2 (10 min) for surface sterilization. Afterwards they were washed with distilled water three times. Furthermore the seeds were germinated in the greenhouse on filter paper moistened with saturated $CaSO_4$ solution. Eight days after sowing, five seedlings with similar root length were transferred to a 1750 ml pot filled with Perlite (Knauf Perlite GmbH, Dortmund, Germany) and moistened with distilled water. The substrate Perlite (Knauf Perlite GmbH, Dortmund, Germany) was rinsed with distilled water before use on a 1 mm sieve to obtain a uniform substrate of 1-3 mm and to prevent cation accumulation on the fraction <1 mm in advance. Furthermore the substrate was autoclaved at 121 °C for 20 min.

The pots were filled with 7.5-cm layer of perlite. Then a 2-cm layer of 10% v/v mycorrhizal inoculum with perlite was introduced and covered with 1.5 cm of perlite. The mycorrhizal fungi used was a Chinese Glomus mosseae isolate (BEG 189), provided by Gu Feng, CAU, China. In non-mycorrhizal (NAM) treatments sterilized mycorrhiza inoculum was applied (autoclaved at 121 °C for 20 min). In addition, the drain of non-sterilized mycorrhiza inoculum was filtered (589/3 blue ribbon paper filter, Schleicher & Schuell Bioscience GmbH, Dassel, Germany) and added to the NAM pots. This was done in an attempt to supply similar amounts of nutrients and microorganisms except AM fungi to all treatments.

The tops of the pots were covered with black/white plastic film (white side up) to reduce evaporation and algae growth. When the first true leaf emerged, the seedlings were supplied twice a day with a fifth-strength Hoagland solution at pH 5.6 (Hoagland and Arnon, 38) with MES buffer added. From the fifth leaf stage onwards, 14 days after planting, the plants were watered twice a day with a third-strength modified Hoagland solution. Sufficient solution was applied so that at least one-third of the applied amount of solution drained from the pots.

Nitrogen was provided at ammonium:nitrate ratios of 6:94 (low), 43:57 (intermediate), or 100:0 (high). The nutrient solutions consisted of the following macronutrients (mM) NO_3^- 6.6, 3.3, 0 and NH_4^+ 0.4, 4.3, 15 depending on the N-treatment; K^+ 2.9; PO_4^{3-} 0.4; Mg^{2+} 1.6; SO_4^{2-} 1.6, 1.6, 1; Ca^{2+} 3.6; Cl^- 4.33,12.1, 26.0; and micronutrients (µM) Fe^{2+} 5.5; Mn^{3+} 2.5; Zn^{2+} 0.4; BO_3^{3-} 18; Cu^{2+} 0.3; MoO_4^{2-} 0.2. A pH of 5.6 was maintained by adding MES-buffer at 0.7mM and NaOH. The compounds used for preparation of the nutrient solution were $Ca(NO_3)_2$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, KH_2PO_4, $MgSO_4$,

$MgCl_2$, KCL, $CaSO_4$, $CaCl_2$, MES-buffer, Fe DTPA, $MnSO_4$, $ZnSO_4$, H_3BO_3, $CuSO_4$, H_2MoO_4.

The difference in N as NO_3^- and S supply has its origin by calculating the MES buffer ($C_6H_{13}NO_4S$**)** into the nutrient solution as a nutrient salt. In the end of the experiment, the buffer was seen as an inert substance. The high ammonium:nitrate ratio was chosen as an extreme treatment, because a mistake was done in the calculation of the molarities of the ammonium salts, $(NH_4)_2SO_4$ and NH_4Cl. Anyway, the nutrient solution remained unchanged during the whole experiment. The plants were rinsed with distilled water once a week, to prevent accumulation of supplied solutes on the perlite.

The experiment was carried out between 24 July to 6 Oct. 2004 in a greenhouse facility at Großbeeren, Germany (long. 13°20´E; lat. 51°22´N). Average air temperature in the greenhouse during this period was 22 °C (max. 36 °C) during the day and 18 °C (min. 13 °C) at night. Relative humidity was on average 60% during the day and 70% at night. The daily (13 h) mean light intensity (PAR) was at 6.8 $mol \cdot m^{-2}$ (max. 660 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The pots were arranged in a completely randomized design.

5.3.2 Harvest and plant analysis

Leaves were counted on each plant 10 weeks after planting. Harvest was carried out 14 weeks after planting in the morning after 2h light. Shoots were separated from the roots. The shoots were cut lengthwise, to provide samples for the analysis of total pyruvic acid (PA) and soluble solid compounds (SSC) from fresh material. For sugar analysis (glucose, fructose (reduced sugars, RS) and sucrose), another sample was separated into bulbs and leaves and frozen at -20 °C. The remainder of the shoots was dried at 60 °C for two days, and dry weight (DW) recorded. Shoots were ground in a centrifugal grinder with a 0.25-mm sieve and used for nutrient analysis of total N, NO_3^-, P, and S.

Samples of the roots were taken by cutting the root and substrate with sharp knifes into equal portions. Roots were separated from the substrate by rinsing with running cold water using a set of sieves (smallest sieve size 1 mm). Roots were then cut in 1-cm pieces and stored in 10% isopropanol for determination of mycorrhizal colonization. Total root dry weight was not determined.

Shoot samples were dry ashed and dissolved in 18.5% HCl. Phosphorus was analyzed photometrically with an EPOS-analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar Vario EL, Hanau, Germany). Nitrate concentration was measured potentiometricly with a NO_3^- ionplus Sure-Flow® electrode (Orion-Research, Beverly; USA) as described in the manufacturer's manual. Total S was analyzed in an elementary analyzer (high temperature oxidation) and detected with NDIR (multi EA 2000, Analytik Jena AG, Germany).

For sugar analysis, 10 g of frozen shoot material was homogenised with water and boiled to denature the sugar enzymes. In the filtrate, sucrose, glucose and fructose were determined by an enzymatic reaction (test kit of r-biopharm, Roche, Darmstadt, Germany) and measured with a spectrophotometer at 340 nm (SPEKOL 221, Carl Zeiss, Jena, Germany).

Soluble solids compounds (SSC) were determined by extracting the juice from at least 10 g of fresh sample in a household centrifuge juicer and subsequent measurement with a hand-held refractometer (digital refractometer PR-1, Atago, Tokyo, Japan). The measurement is based on the capacity of dissolved sugar in a juice to deviate light and gives the proximate sugar total content in °Brix (Ahlers, 01; OECD, 02).

Total PA was analysed in 15 g of ruptured shoot tissue using the method modified and described by Schwimmer and Weston (61), Randle and Bussard (93), and Ketter and Randle (98). The fresh shoot tissue was homogenized with 30 ml distilled water, after 20 min mixed with 5% trichloroacetic acid solution (1:1) and stored for 1h. The filtrate was mixed with the indicator 0.0125% 2,4-dinitrophenylhydrazine in 2N HCl, then alkalined with 0.6 N NaOH, and the measured transmission at 420 nm. Background levels of PA of intact onion tissues were assumed to be negligible and constant (Yoo and Pike, 01), so that background PA was not measured.

Mycorrhizal colonization of roots was determined following the method of Koske and Gemma (89). Roots were cleared with 10% KOH, acidified with 2N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization was determined in the stained samples using a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 50x (grid line intersection method, Giovannetti and Mosse, 80).

5.3.3 Statistics

Data (n = 5) were subjected to a two-way analysis of variance, with inoculation and ammonium:nitrate ratios in supply as experimental factors. Within each factor, differences between means were tested by the Tukey method (N speciation) or by t-tests (AM inoculation). Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, USA) software.

5.4 Results

5.4.1 Mycorrhizal colonization and plant growth

Roots were colonized by AM fungi in treatments with live mycorrhizal inoculum. The apparent root length colonization rate was low (0.8 to 1.3% colonization) and was not

significantly affected by N speciation treatment. Not inoculated plants were free of mycorrhizal colonization.

N speciation affected the number of leaves and shoot dry weight (Tab. 11). At the high ammonium:nitrate ratio, shoot growth was significantly suppressed and leave tips were wilting, especially in the NAM treatment. The number of leaves and shoot dry weight in the other two N speciation treatments did not differ significantly except for an increase in dry weight of the NAM plants at the low ammonium:nitrate ratio compared to the intermediate ratio.

Tab. 11: Effect of N speciation (N) and AM treatments (m) on leaf number (10 weeks after planting) and on shoot dry weight and pyruvic acid (PA) concentration (14 weeks after planting) of *Allium fistulosum*. Plants were supplied with three different ammonium:nitrate ratios and either inoculated with AM (AM) fungi or non-inoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Differences between the levels of AM treatments are denoted as significant (*) or non-significant (^{ns}) (t-test). Differences between the levels of N speciation are denoted with capitalized letters either *italicized* (NAM) or non-italicized (AM), as determined by Tukey tests (P<0.05). Values are means of 5 observations and standard error of mean (SE) (±).

	Leaf number n° pot^{-1}	Dry weight g pot^{-1}	PA µmol pot^{-1}
NH4 : NO3 high			
NAM	3.4 ± 0.2A	3.2 ± 0.5*A*	217 ± 23*A*
AM	4.1*± 0.1A	7.4*± 0.2A	448*± 30A
NH4 : NO3 intermediate			
NAM	4.3 ± 0.2*B*	7.1 ± 0.4 *B*	481 ± 33*B*
AM	4.8*± 0.1B	9.5*± 0.5B	741*± 91AB
NH4 : NO3 low			
NAM	4.8 ± 0.1*B*	9.9 ± 0.5*C*	629 ± 39*C*
AM	4.8^{ns}± 0.1B	10.8^{ns}± 0.8B	872^{ns}± 160B
P (N)	*<0.001*	*<0.001*	*<0.001*
P (m)	*<0.001*	*<0.001*	*<0.001*
P (N x m)	*0.017*	*0.022*	0.982

The numbers of leaves 10 weeks after planting and dry weight of the shoots at harvest were also affected significantly by colonization with AM fungi (Tab. 11). Colonization with AM fungi increased shoot growth at the high and intermediate ammonium:nitrate ratios, but not when NO_3^- was dominant N speciation treatment.

5.4.2 pH of the nutrient solution and the drain

The pH of the drain was elevated, compared to that of the applied solution, in all treatments at the beginning of the experiment when the plants were young (Fig. 8). Towards the end of the experiment, the pH of the drain in the high ammonium:nitrate

ratio clearly decreased. The drain from the ratio of intermediate was slightly acidified, whereas drain pH at the low ammonium:nitrate ratio remained higher than that of the applied solution (Fig. 8).

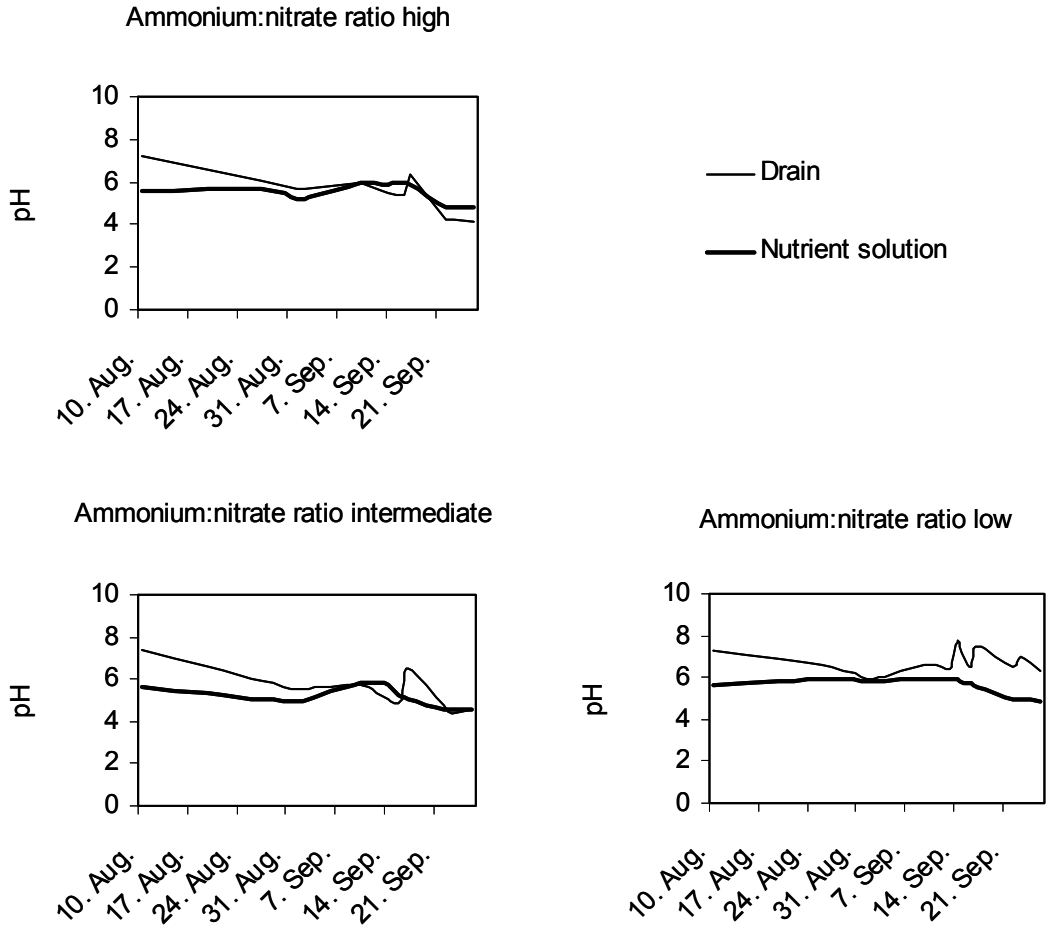


Fig. 8: Effect of N speciation on pH of the drain during the experiment.

5.4.3 Mineral elements

Shoot N concentration was highest at the high ammonium:nitrate ratio (Tab. 12). Shoot S concentration was also significantly influenced by N-speciation treatment, with the highest concentration in NAM plants at the high ammonium:nitrate ratio. Shoot N/S ratio was highest in AM plants at the high ammonium:nitrate ratio. Shoot P concentration increased significantly with increased rate of NH_4^+ supply. Shoot NO_3^- concentration was significantly enhanced at the low ammonium:nitrate ratio.

Tab. 12: Effect of N speciation (N) and AM treatments (m) on shoot N/S-ratio, N, NO_3^-, S, and P concentration and content 14 weeks after planting of *Allium fistulosum*. Plants were supplied with three ammonium:nitrate ratios and inoculated with AM (AM) fungi or non-inoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Differences between the levels of AM treatment are denoted as significant (*) or non-significant (^{ns}) (t-test). Differences between the N speciation are denoted with capitalized letters either *italicized* (NAM) or non-italicized (AM), as determined by Tukey tests (P<0.05). Values are means of 5 observations and SE (±).

	Shoot element concentration g [kg DW]$^{-1}$				
	N	S	P	NO_3	N/S-ratio
NH_4 : NO_3 high					
NAM	46.2 ± 1.7*B*	5.6 ± 0.1*B*	5.1 ^{x}	2.9 ^{x}	8.2 ± 0.1*A*
AM	47.8^{ns}± 1.2B	4.8*± 0.1A	5.2 ± 0.1C	5.0 ± 0.6A	9.9*± 0.2B
NH_4 : NO_3 intermediate					
NAM	41.6 ± 0.8*A*	5.0 ± 0.2*A*	4.3 ± 0.3*B*	3.7 ± 0.3*A*	8.4 ± 0.3*A*
AM	42.1^{ns}± 0.4A	4.8^{ns}± 0.1A	4.4^{ns}± 0.2B	5.3*± 0.4A	8.7^{ns}± 0.2A
NH_4 : NO_3 low					
NAM	41.2 ± 0.4*A*	5.2 ± 0.2AB	3.8 ± 0.2*A*	6.0 ± 0.5*B*	7.9 ± 0.3*A*
AM	42.3^{ns}± 1.2A	5.2^{ns}± 0.2A	3.7^{ns}± 0.1A	7.1^{ns}± 0.6B	8.1^{ns}± 0.3A
P (N)	*<0.001*	*0.049*	*<0.001*	*<0.001*	*0.003*
P (m)	0.199	*0.014*	0.788	*<0.001*	*0.002*
P (N x m)	0.853	*0.024*	0.931	0.748	*0.018*

	Shoot element total content mg pot^{-1}			
	N	S	P	NO_3
NH_4 : NO_3 high				
NAM	127 ± 37*A*	15 ± 4*A*	25 ^{x}	14 ^{x}
AM	354*± 13A	36*± 1A	38 ± 1A	36 ± 4A
NH_4 : NO_3 intermediate				
NAM	297 ± 18*B*	35 ± 2*B*	30 ± 1A	26 ± 3*A*
AM	416*± 19AB	48*± 3AB	44*± 3A	52*± 4A
NH_4 : NO_3 low				
NAM	390 ± 23*B*	49 ± 3*C*	36 ± 2A	56 ± 4*B*
AM	455^{ns}± 34B	57^{ns}± 6B	40^{ns}± 3A	76^{ns}± 8B
P (N)	*<0.001*	*<0.001*	0.186	*<0.001*
P (m)	*<0.001*	*<0.001*	*<0.001*	*<0.001*
P (N x m)	*0.032*	0.377	0.180	0.846

^{x} single measurements of a combined sample of five replications

Colonization with AM fungi had no influence on shoot N or P concentration, but it decreased shoot S concentration at the high ammonium:nitrate ratio. Shoot NO_3^- concentration was increased by AM colonization at the high and intermediate ammonium:nitrate ratios. Shoot N/S ratio was significantly enhanced by AM colonization at the high ammonium:nitrate ratio.

Shoot N total content was significantly lower at the high ammonium:nitrate ratio compared to the other two ammonium:nitrate ratios, irrespectively from AM colonization (Tab. 12). The same pattern applied to the S total contents of AM plants. The S total content of NAM plants was significantly different between the three ammonium:nitrate ratios, with the highest S total content at the high ammonium:nitrate ratio. Shoot P was not significantly influenced by N-speciation. Shoot NO_3^- total content was significantly higher at the low ammonium:nitrate ratio than in the other two ammonium:nitrate ratios.

Mycorrhizal plants had increased shoot N, S, P, and NO_3^- total contents at the high and intermediate ammonium:nitrate ratios compared to corresponding NAM plants.

5.4.4 Sucrose, reducing sugars, pyruvic acid, and soluble solid compounds

The concentration of reducing sugars (glucose and fructose) in the green part of the shoot (leaves) was significantly increased at the high and intermediate ammonium:nitrate ratios compared to plants at the low ammonium:nitrate ratio, at 8.1 ± 0.2b, 7.9 ± 0.1b and 6.8 ± 0.1a g [kg DW]$^{-1}$ (means of AM and NAM plants), respectively, but not by AM colonization. In contrary, sucrose concentration was significantly increased by AM colonization, from 1.6 g [kg DW]$^{-1}$ in NAM plants to 2.4 g [kg DW]$^{-1}$ in AM plants, but were not influenced by the different ammonium:nitrate ratios. The concentrations of reducing sugars and sucrose in the bulbs were not significantly different between the treatments.

Plant SSC were not affected by different ammonium:nitrate ratios or by AM colonization. Mean SSC concentration was determined as 4.2 °Brix.

Shoot PA concentration, a measure of gross flavor intensity, did not respond significantly to N-speciation or AM treatment (data not shown). Shoot PA total content was enhanced by increasing the rate of NO_3^- supply, and it was also enhanced by AM colonization at the high and intermediate ammonium:nitrate ratios (Tab. 11). Shoot PA total content of AM plants at the intermediate ammonium:nitrate ratio was not significantly different from that observed in AM and NAM plants of the high NO_3^- treatment.

5.5 Discussion

5.5.1 Colonization

All inoculated plants became mycorrhizal in the present experiment, but apparent root colonization rates remained low compared to earlier observations with *Allium* plants. The low root colonization rates may be related to the fact that after 14 weeks of growth, the oldest roots had begun to break down, and the early colonization was no longer visible. Additionally, the nutrient solution supported the plants with sufficient nutrients, especially with P. High P supply can depress mycorrhizal root colonization even if photon irradiance is high (Son and Smith, 88; Smith and Gianinazzi-Pearson, 90; Pearson JN et al., 91). High P within the roots of *Allium cepa* L. might influence the rate of spread of the fungus and the growth of the extraradical mycelium (Sanders, 75).

Plant colonization with AM fungi in the present experiment were more tolerant to high NH_4^+ fertilization, which was accompanied by low pH in the medium. Hyphae of at least some mycorrhizal fungi have a higher ability to take up NH_4^+ than NO_3^- (Hawkins et al., 00). Recent experiments have shown that both NO_3^-- and NH_4^+-N is assimilated into arginine at the tip of the hyphae, transported in the hyphae, and transferred probably as ammonia (NH_3) at the fungus-plant interface (Govindarajulu et al., 05). Thus, in mycorrhizal plants the acidification related to NH_4^+ uptake does not occur concentrated in the immediate rhizosphere, but also in the hyphosphere, so root damage due to acidification may be reduced in mycorrhizal plants.

5.5.2 The pH of the nutrient solution and the drain

An acidification of the rhizosphere takes place when NH_4^+ is incorporated into the plant and, in exchange, H^+ ions are excreted (Marschner, 95). The MES buffer used in this experiment was not sufficiently strong to prevent these changes. It is possible that the volume of the solution used at each irrigation interval was too small to buffer the H^+ ions efficiently. Previous experiments with MES buffer (Bugbee and Salisbury, 85; Miyasaka et al., 88; Hawkins et al., 00) used either hydroponic systems or a steady drip irrigating system.

5.5.3 Number of leaves and shoot dry weight

In the present experiment, plant growth was inhibited by increased rates of NH_4^+ supply, but this inhibition was to some extent compensated for by AM colonization. A similar result was also observed by Bago and Azcón-Aguilar (97) in *Allium cepa*.

Hawkins and George (Hawkins and George, 01) found that although NO_3^- fed wheat plants were larger than NH_4^+ fed plants, colonization with AM increased shoot dry weight in both N fertilization treatments.

Ammonium ions are in most soils readily adsorbed by soil particles, but in nutrient solutions growth depression caused by sole NH_4^+ supply (>1 mM N) can be distinct (Marschner, 95). Whereas NO_3^- can be stored in vacuoles without detrimental effect, NH_4^+ and in particular its equilibrium partner NH_3 are toxic to the plant at quite low concentrations. The concentration of NH_4^+ in the cytoplasm is usually lower than 15µM. The main pathway of detoxification within the plant is the formation of amino acids, amides and related compounds. To compensate for the change in charge, protons are released into the rhizosphere, resulting in a decline in rhizosphere pH that can affect root growth and, as a consequence, cause leaf wilting (Marschner, 95; Claussen and Lenz, 95). On the other hand, Date et al. (05) found that addition of chlorid (Cl) at 8.5×10^{-3} mM with 0.67 mM NH_4^+ to a nutrient solution caused wilting of lettuce leaf tips, because of the formation of chloramines in the solution. It has been observed that nutrient solutions containing Cl up to 5 mM did not have toxic Cl effects on most plants (V. Römheld, personal communication). With increasing rate of NH_4^+ supply, Cl concentrations in the nutrient solution of the present experiment were 4, 12 and 26 mM. Leaf wilting occurred at the highest NH_4^+ supply ratio only, suggesting an effect of high Cl supply. Such wilting was not observed in AM plants that were perhaps better protected against Cl toxicity. Another reason for decreased growth with increased NH_4^+ supply may be the occurrence of NO_3^- deficiency. Nitrate deficiency was apparent in a study where 2mM NH_4^+ was the sole N supply (Walch-Liu et al., 00).

Even at mixed ammonium:nitrate supply external NH_4^+ strongly suppresses net uptake of NO_3^- (Kronzucker et al., 99a). One explanation for the suppression of net NO_3^- influx by NH_4^+ in the plasma membrane could be the inhibition of the inducible high-affinity NO_3^- transport system (Kronzucker et al., 99a). These earlier observations were supported by the present findings on shoot NO_3^- concentration. Shoot NO_3^- concentrations, but not shoot N concentrations were decreased in the intermediate ammonium:nitrate ratio compared to a low ratio.

Other inhibiting effects may also be associated with supply of NH_4^+ as the sole N source: uncoupling of photophosphorylation, lack of carbohydrates or nutrients, and impairment of water status (Walch-Liu et al., 00). These effects are unlikely to have been important in the present study, as the plants were well provided with light and nutrients, and had no drought stress.

5.5.4 Mineral elements

Compared to standard values for leaves of *Allium cepa* (Bergmann, 93), element concentrations in the present experiment indicated sufficient supply of N and P.

As expected, shoot NO_3^- concentrations were significantly reduced with increasing ammonium:nitrate ratio. The decreased shoot NO_3^- concentrations at the high and intermediate ammonium:nitrate ratios indicate that intercellular NO_3^- was immediately metabolized in the plant, whereas at the low ammonium:nitrate ratio, surplus NO_3^- was stored in vacuoles. Additionally, AM colonization significantly enhanced shoot NO_3^- concentration (Tab. 12). This effect is surprising in view of the fact that hyphae of mycorrhizal fungi may prefer NH4+ as an N source. However, mycorrhizal fungi can also take up NO3- (Govindarajulu et al., 05). Alternatively, NH4+ uptake by hyphae may downregulate root NH4+ uptake, leading to higher shoot NO3- concentrations and total contents in mycorrhizal plants. Anyway, in cases where shoot NO3- concentrations should be kept low, non-mycorrhizal plants may be preferable to mycorrhizal plants.

The increased shoot N and S concentrations at the high ammonium:nitrate ratio compared to the other ratios were probably due to the decreased shoot dry weight in this treatment. This was confirmed by the low N and S total content in that treatment (Tab. 12). In contrast to S concentrations, P concentrations increased with increasing rate of NH_4^+ supply. In general, NH_4^+ absorption into roots may support the uptake of anions to maintain ionic balance in the plant (Marschner, 95; Van Beusichem et al., 88). This effect was evident for PO_4^{3-}, but not for SO_4^{2-} uptake. Sulfate was supplied at much higher concentrations than PO_4^{3-} in the nutrient solution, while final S and P concentrations in the shoot were not distinctly different (Tab. 12). Thus, plant P accumulation probably depend more on active uptake process than plant S accumulation.

Mycorrhizal colonization did not result in increased shoot N or P concentrations. N and P supply to roots with the nutrient solution was sufficient, so that additional uptake via hyphae was probably small and not detectable. For shoot S concentrations, an AM effect was observed at the high ammonium:nitrate ratio. In this case the S concentration was significantly higher in the NAM treatment, probably due to restricted shoot growth in those plants.

5.5.5 Sugars, PA and SSC

Observed concentrations of reducing sugars in leaves correspond with those found by Randle and Bussard (93) and Randle (92a) in *Allium cepa*, whereas SSC was slightly lower than those of Randle (92a) and Randle and Bussard (93). The SSC are usually used to control the degree of maturation and quality in fruits and vegetables (Ahlers, 01; OECD, 02). It is an approximate value of the sugar concentration of the juice.

Concentrations of reducing sugars and sucrose as found in the present study are part of the *Allium* flavor. Nitrogen and soluble sugars are also the main substances required for leaf growth and expansion (Walch-Liu et al., 00). After transport of amino acids to the leaves, their metabolism requires a substantial consumption of fixed carbon (C) (Raab and Terry, 95). It has been frequently stated that root growth and therefore whole plant growth of NH_4^+-fed plants is restricted by low availability of carbohydrates due to excessive consumption of soluble sugars for NH_4^+ assimilation (detoxification) in root tissue (Kafkafi, 90). In contrast to expectations in the present study shoot glucose and fructose concentrations were highest in the treatment with dominant NH_4^+ supply. It is likely that Cl toxicity, low pH or nitrate deficiency restricted shoot growth more than photosynthesis in this treatment, so that some sugars could accumulate.

In leaves of AM-colonized plant, sucrose concentration was higher than in leaves of non-mycorrhizal plants. Thus, consumption of hexoses, the metabolite of hydrolization of sucrose by an acid invertase (Schubert et al., 03), by mycorrhizal fungi was probably limited, in line with low root colonization rate.

Observed PA concentrations were consistent with the results of Randle (92a) and Randle and Bussard (93) in onion. His results had shown that PA concentration increase with S concentration in the plant. This could not be confirmed in the present experiment. It is likely that the S supply with the nutrient solution was sufficient to meet plant S demand in all treatments, thereby masking possible effects of N speciation and AM treatment on shoot S and PA concentration. Under these circumstances, highest shoot PA yield was obtained in mycorrhizal plants and at dominant nitrate supply (Table 1).

In conclusion, this experiment demonstrated that (a) bunching onion shoot dry weight decreased with increasing rate of NH_4^+ supply, but the decrease was mitigated or eliminated by AM colonization, (b) AM colonization increased shoot dry weight at high rate of NH_4^+ supply (low substrate pH), and (c) pyruvic acid shoot yield was increased at higher rate of NO_3^- supply and by mycorrhizal colonization.

Allium fistulosum performed best at an low ammonium:nitrate ratio, but when supported by AM fungi it produced similar amounts of pyruvic acid at an intermediate ammonium:nitrate ratio.

Chapter 6

6 Influence of sulfur supply, nitrogen speciation, and arbuscular mycorrhizal colonization on growth and composition of Chinese chive

6.1 Abstract

Allium species form compounds during their growth that are interesting for the consumer because of their health benefits and flavor. Their concentrations are influenced by S and N. To test those relations three factors were used in the present two experiments on Chinese chive [*Allium tuberosum* Rottler ex Sprengel]: 1. Increasing S concentrations in the substrate, 2. three different ratios of ammonium and nitrate in combination with AM fungi. These factors were tested regarding dry weight, nutrient composition (N, P, Mg, S, NO_3^-), single sugars (glucose, fructose, sucrose), total soluble solid and health related organosulfur compounds, measured indirectly as pyruvic acid. In the first experiment the supply of low S concentrations to the substrate resulted in deficiency symptoms, but in similar growth at intermediate and high S supply. The increasing S concentrations in the substrate increased shoot S concentrations and pyruvic acid concentrations, but had no influence on the soluble solid compounds.

In the second experiment inoculation with a commercial inoculum resulted in colonization rates of 43% of total root length for the highest NO_3^- supply, decreasing with increasing NH_4^+ supply, but had only a low impact on the plant growth and composition. Mycorrhizal colonization increased shoot S concentrations, but did not significantly increase shoot dry weight, shoot N, P, K, Mg and NO_3^- concentrations, sugar, or pyruvic acid concentrations. In contrast, the N speciation had a high impact. Plants grown at an ammonium:nitrate ratio of 50:50 did not show any NH_4^+ toxicity symptoms which showed in equally shoot dry weight as at a ratio of 5:95. Additionally the N and pyruvic acid concentrations were increased at an ammonium:nitrate ratio of 50:50 compared to the ratio of 5:95. Therefore, we conclude that the supply of an ammonium:nitrate ratio of 50:50 is preferential to dominant NO_3^- fertilization for *Allium tuberosum* to produce a high yield of health related organosulfur compounds.

6.2 Introduction

The introduction of chapter 4 gave a summary of the recent findings and coherences on the plant quality regarding the health related organosulfur compounds of Allium

species. In chapter 4 it has been speculated that the effect of N speciation may have been masked by a high S supply. Therefore a second experiment has been conducted with reduced S supply using Chinese chive as a test plant.

Additionally an experiment has been included that investigated the concentration of organosulfur compounds in *Allium tuberosum* as a function of S supply. Earlier studies have shown that the level of pyruvic acid in onion juice correlates with flavor compound pungency (Schwimmer and Weston, 61). The formation of pungent compounds and sugars (Randle, 92b; Randle and Bussard, 93; McCallum et al., 05) and especially health related organosulfur compounds (Keiss et al., 03), may be increased in *Allium* plants by an increased S supply. Many investigations have been conducted already with *Allium* species, particularly involving garlic and onion, but not Chinese chive. Chinese chive is an important ingredient in Asian cooking (Mau et al., 01) belongs to the four *Allium* species most extensively grown in China (Peiwen et al., 94).

The objective of our study was to determine the effect of (a) an increasing S supply, (b) different ammonium:nitrate ratios, (c) AM colonization on the shoot dry matter, shoot nutrient concentrations (NO_3^-, total N, P, and S), shoot composition (soluble solid compounds, and separately glucose, fructose, and sucrose) and pyruvic acid as indicator for organosulfur compounds of Chinese chive *Allium tuberosum*, and (d) exploration for growth conditions that increase yield of organosulfur compounds.

6.3 Material and Methods

6.3.1 Germination

Seeds of Chinese chive, *Allium tuberosum* Rottler ex Sprengel (Schnittknoblauch, Hild, Marbach, Germany), were suspended in water with 10% H_2O_2 (10 min) for surface sterilization. Afterwards they were washed with distilled water three times. Furthermore the seeds were germinated in the greenhouse or climate chamber on filter paper moistened with saturated $CaSO_4$ solution.

6.3.2 Substrate preparation

The substrate Perlite (Knauf Perlite GmbH, Dortmund, Germany) was rinsed with distilled water on a 1 mm sieve to obtain a uniform substrate of 1-3 mm and to prevent cation accumulation on the fraction <1 mm. The substrate was autoclaved at 121 °C for 20 min. A top layer of gravel on each pot reduced evaporation and algae growth.

6.3.3 Experimental procedure and statistical analysis

6.3.3.1 Experiment 1

Eight days after sowing four seedlings with similar root length were transferred to a 1 L pot filled with Perlite and moistened with distilled water. Each treatment comprised five replicates. When the first true leave emerged, the seedlings were supplied twice a day with a fifth-strength modified Hoagland solution at pH 5.6 (Hoagland and Arnon, 38). From the seventh leaf stage onwards the plants were watered twice a day with a third-strength modified Hoagland solution. Enough solution was applied so that at least one-third drained. The ammonium:nitrate ratio was kept at 5:95. The sulfur (S) concentrations were adjusted to low (0.02 mM), medium (0.2 mM) and high (2 mM) levels. The 0.02 mM nutrient solution was lowered to 0 nine weeks after transplantation. The nutrient solutions consisted of the following macronutrients (mM): SO_4^{2-} 0.0, 0.2, 2.0 depending on the S treatment; NO_3^- 7.1; NH_4^+ 0.4; K^+ 3.0; PO_4^{3-} 0.4; Mg^{2+} 1.7; Ca^{2+} 3.8; and micronutrients (µM): Fe^{2+} 5.6; Mn^{3+} 2.6; Zn^{2+} 0.4; BO_3^{3-} 18.8; Cu^{2+} 0.3; MoO_4^{2+} 0.2; Cl^- 3340, 3012, 5.27. A pH of 5.6 was maintained by adding NaOH.

The compounds used for preparation of the nutrient solution were $Ca(NO_3)_2$, KNO_3, KH_2PO_4, $MgSO_4$, $MgCl_2$, $CaSO_4$, $CaCl_2$, Fe DTPA, $MnCl_2$, $ZnSO_4$, H_3BO_3, $CuSO_4$, H_2MoO_4. The pots were rinsed with distilled water once a week, to prevent an accumulation of solutes on the substrate.

The experiment was carried out in summer in a greenhouse facility at Großbeeren, Germany (long. 13°20´E; lat. 51°22´N). Average air temperatures in the greenhouse during this period were 23 °C (max. 36 °C) during the day and 19 °C (min. 14 °C) at night. The relative humidity was on average 60% during the day and 70% at night. The daily (14h) mean light intensity (PAR) was at 6.6 $mol \cdot m^{-2}$ (max. 660 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during the day. The pots were arranged in a completely randomized design.

Data were subjected to a one-way analysis of variance (n = 5), with S supply as experimental factor, followed by a Tukey test. Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, USA) software.

6.3.3.2 Experiment 2

Ten days after sowing eight seedlings with similar root length were transferred to a 1.3 L pot filled with Perlite and moistened with distilled water. This time each treatment comprised four replicates.

The pots were filled with 2-cm layer of perlite. Then a 3-cm layer of 10% v/v arbuscular mycorrhizal inoculum (AM) with perlite was introduced and covered with 5.5-cm

layer of perlite. As mycorrhiza inoculum Plantworks inoculum was used (TerraVital Hortimix comprising *G. mosseae, G. intraradices, G. claroideum* and *G. microaggregatum*, >50 infective units per ml inoculum. Plantworks Ltd., Heeley Close, Sittingbourne, Kent, UK).

In non-mycorrhizal (NAM) treatments sterilized Plantworks inoculum was applied (autoclaved at 121°C for 20 min). In addition, the drain of non-sterilized Plantworks inoculum was filtered (589/3 blue ribbon paper filter, Schleicher & Schuell Bioscience GmbH, Dassel, Germany) and added to the NAM pots. This supplied similar amounts of nutrients and micro-organisms except AM to all treatments.

When the first true leave emerged, the seedlings were supplied twice a day with a tenth-strength modified Hoagland solution at pH 5.6 (Hoagland and Arnon, 38) with a MES buffer added.

From the second leave stage onwards, 14 days after planting, the plants were watered twice a day with a third-strength modified Hoagland solution. Enough solution was applied so that at least one-third drained. Nitrogen was provided at an ammonium:nitrate ratio of 95:5, 50:50, and 5:95. The nutrient solution consisted of the following macronutrients (mM) NO_3^- 0.4, 3.7, 7.0 and NH_4^+ 7.0, 3.7, 0.4 depending on the N speciation treatment; K^+ 2.9; PO_4^{3-} 0.4; Mg^{2+} 1.6; SO_4^{2-} 0.2; Ca^{2+} 3.6; and micronutrients (µM) Fe^{2+} 5.5; Mn^{3+} 2.5; Zn^{2+} 0.4; BO_3^{3-} 18; Cu^{2+} 0.3; MoO_4^{2+} 0.2; Cl^- 16666, 11434, 4276. A pH of 5.6 was maintained by adding MES-buffer at 0.7mM and NaOH. The compounds used for preparation of the nutrient solution were $Ca(NO_3)_2$, KNO_3, NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, KH_2PO_4, $MgSO_4$, $MgCl_2$, KCL, $CaSO_4$, $CaCl_2$, MES-buffer, Fe DTPA, $MnSO_4$, $ZnSO_4$, H_3BO_3, $CuSO_4$, H_2MoO_4. The pots were rinsed with distilled water once a week, to prevent an accumulation of supplied solutes on the substrate.

The experiment was carried out in a climate chamber. The average air temperatures in the climate chamber during the day (14 h) were 17 °C and 15 °C during the night (10 h). The relative humidity was 70% during the day and 80% at night. The daily (14h) mean light intensity (PAR) was at 28.2 mol·m^{-2}. Light was provided by lamps (Agro Son T 400, Phillips, Hamburg, Germany). The pots were arranged in a completely randomized design.

The data were subjected to a two-way variance analysis (n = 4), utilizing inoculation and ammonium:nitrate ratios in the supply as experimental factors. This was followed by a Tukey test comparing means wthin each factor. Data were analyzed using Statistica 6.1 (StatSoft, Tulsa, OK, USA) software.

6.3.4 Harvest

Plants were harvested in the morning after 2h light in the first experiment and after 4h darkness in the second experiment. Leaves were counted during the second experiment only. The shoots were cut 1 cm above ground at 6, 14 (Expt. 1) and 13 (Expt. 2) weeks after the initial planting. They were cut into pieces for direct analysis of total pyruvic acid (PA) and soluble solid compounds (SSC). 10 g of the plant material were frozen at -20 °C for sugar analysis (sucrose, glucose, and fructose). The remainder was dried at 60 °C for two days, and its dry weight (DW) recorded. Shoots were ground in a centrifugal grinder with a 0.25 mm sieve and analyzed for total S, N, P (Exp. 1) and additionally Mg and NO_3^- (Exp. 2).

For root dry weight determination substrate samples of defined volume were taken with a cylinder. Additionally root samples (Exp. 2) were separated from the substrate by rinsing with running cold water and a set of sieves (smallest sieve size 1 mm). These randomly taken roots were cut in 1-cm pieces and stored in 10% isopropanol for determination of mycorrhizal colonization.

6.3.5 Analysis

Shoot samples were dry ashed and dissolved in 18.5 % HCl. Phosphorus and Mg were analysed photometrically with an EPOS-analyzer 5060 (Eppendorf, Hamburg, Germany). Nitrogen was determined after dry oxidation by the DUMAS method (Elementar Vario EL, Hanau, Germany). Nitrate concentration was measured potentiometricly with a nitrate ionplus Sure-Flow® electrode (Orion-Research, Beverly; USA). Total S was analyzed in an elementary analyzer (high temperature oxidation) and detected with non-dispersive infrared technique (NDIR) (multi EA 2000, Analytik Jena AG, Germany).

For sugar analysis 10 g of frozen shoot material was homogenised with water and boiled to denature the sugar enzymes. In the filtrate, sucrose, glucose and fructose were determined by an enzymatic reaction (test kit of r-biopharm, Roche, Darmstadt, Germany) and measured with a spectrophotometer at 340 nm (SPEKOL 221, Carl Zeiss, Jena, Germany).

Soluble solids compounds (SSC) were determined by extracting the juice from at least 15 g fresh sample in a household centrifuge juicer and measured with a hand-held refractometer (digital- refractometer PR-1, Atago, Tokyo, Japan). The measurement is based on the capacity of sugar in a juice to deviate light and gives the proximate sugar content in °Brix (Ahlers, 01; OECD, 02).

Total pyruvic acid (PA) was analyzed in 20 g ruptured shoot tissue using the method described by Schwimmer and Weston (61), Randle and Bussard (93), and Ketter and Randle (98). The fresh shoot tissue was homogenized with 30 ml distilled water, after 20 min mixed with 5% trichloroacetic acid solution (1:1) and left standing for 1h. The filtrate was mixed with the indicator 0.0125% 2,4-dinitrophenylhydrazine in 2N HCl, then alkalined with 0.6 N NaOH, and the measured transmission at 420 nm. Background levels of PA of intact onion tissues were assumed to be negligible and constant (Yoo and Pike, 01), so that background pyruvic acid was not measured.

Mycorrhizal colonization of roots was determined following the method of Koske and Gemma (89). Roots were cleared with 10% KOH, acidified with 2 N HCl, and stained with 0.05% trypan blue in lactic acid. Percentage root length colonization was determined in the stained samples using a microscope (Zeiss, Stemi2000, Göttingen, Germany) at 50x (grid line intersection method) (Giovannetti and Mosse, 80).

6.4 Results

6.4.1 Experiment 1

During the second harvest, (7 Sep. 04) the phenotype of the young leaves on no S in the solution was pale-green, whereas the older leaves were slightly darker green. At 0.2 mM S the leaves were dark green and at 2.0 mM S with a lightly brighter shade.

The dry weight of no sulfate supply was significantly lower and analogously the shoot N concentration significantly higher compared with the higher S supplies (Tab. 1). The shoot P concentration increased with decreasing S supply. Shoot S concentrations showed significant differences between the three S supplies. Because of the opposite uptake of N and S, the N/S ratio rose significantly with decreasing S supply.

The consideration of the nutrient content of each pot (data not shown) showed that shoot N and S contents were lowest at 0.0 mM S supply. At the two higher S supplies the shoots did not differ in their N content, but had the highest S content at the highest S supply. The shoot P content was similar in all cases.

Pyruvic acid concentration increased significantly with increasing S supply and shoot S concentration. The SSC in the 0, 0.2, and 2.0 mM S treatment was at 5.0, 5.7, and 5.6 °Brix, respectively, but not significantly different. The pH of the drain was not measured.

Tab. 13: Effect of S supply (mM S) on shoot dry weight (DW), shoot N, P, S concentration, N/S ratio and pyruvic acid (PA) concentration 14 weeks after planting of *Allium tuberosum*. Plants were supplied with three different S concentrations (0.0, 0.2 and 2.0 mM) at ammonium:nitrate ratio 5:95. Effects of the treatments were tested with a one-way ANOVA. Different letters denote significant differences between means of all treatments as determined by the Tukey test (P<0.05). Values are means of 5 observations and standard error of the mean (SE) (±).

	DW	Element concentration				PA
	g pot^{-1}	g [kg DW]$^{-1}$				µmol [g FW]$^{-1}$
mM S		N	P	S	N/S ratio	
0.0	9.6 ± 0.5a	47.3 ± 0.8b	4.1 ± 0.1c	1.8 ± 0.1a	26.2 ± 0.6c	2.1 ± 0.1a
0.2	12.0 ± 0.4b	42.9 ± 0.3a	3.5 ± 0.1b	3.3 ± 0.2b	13.2 ± 0.5b	2.9 ± 0.2b
2.0	13.0 ± 0.5b	41.6 ± 0.5a	3.2 ± 0.1a	6.2 ± 0.1c	6.7 ± 0.1a	5.0 ± 0.3c
P (S)	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

6.4.2 Experiment 2

6.4.2.1 The pH in the drain

The pH in the nutrient solution with the highest ammonium:nitrate ratio was lowered from 5.6 to nearly 4 while running through the pot, whereas at the lowest ammonium:nitrate ratio the pH was increased up to 8. The an equal ammonium:nitrate ratio the pH remained at 5.6 (Fig. 9).

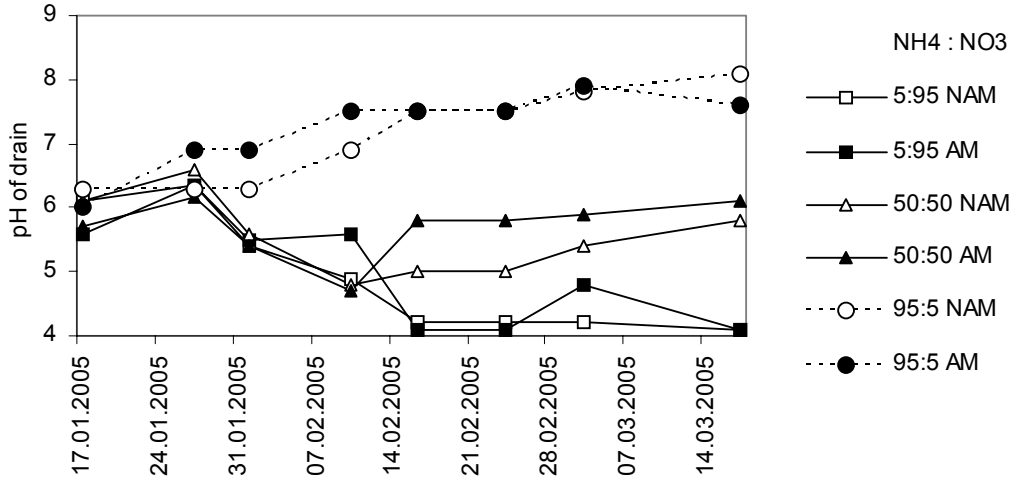


Fig. 9: Effect of N speciation and AM colonization on pH in the drain of the nutrient solution during the experiment with *A. tuberosum*.

6.4.2.2 Root length colonization by arbuscular mycorrhiza

Roots were colonized by AM if treated with live mycorrhizal inoculum only. The highest root length colonization rate 43 ± 14 % was found at an ammonium:nitrate ratio of 5:95, decreasing rapidly with increasing ammonium:nitrate ratio. At the ammonium:nitrate ratio of 50:50 root length colonization was 8 ± 4.2 % and at 95:5 the roots showed no colonization at all.

6.4.2.3 Number and length of leaves, fresh and dry weight of shoots and roots

The number and length of the leaves (shoots) (data not shown), the fresh and dry weight of leaves and dry weight of roots (Tab. 14) were not influenced by AM treatment. The leaves at the ammonium:nitrate ratio 95:5 were light green whereas the older leaves showed wilted tips.

Tab. 14: Effect of N speciation (N) and AM treatments (m) on shoot fresh weight, shoot and root dry weight, S concentration, and S content 13 weeks after planting of *Allium tuberosum*. Plants were supplied with three different ammonium:nitrate ratios and either inoculated with AM (AM) or non-inoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Differences within the factor inoculum are denoted as significant (*) or non-significant (^{ns}), differences between the factor NH_4^+:NO_3^- are denoted with capitalized letters either *italicized* (NAM) or non-italicized (AM) as determined by the T-test or Tukey test (P<0.05), respectively. Values are means of 4 observations and SE (±).

		FW	DW		Concentration	Content
		g pot^{-1}	g pot^{-1}		g kg^{-1}	g pot^{-1}
		shoot	shoot	root	S	S
NH_4:NO_3		Inoc				
95:5	NAM	7.3 ± 0.2*A*	1.4 ± 0.0*A*	2.4 ± 0.1*A*	4.59 ± 0.1*C*	6.6 ± 0.1*A*
	AM	6.0^{ns}± 0.6A	1.3^{ns}± 0.1A	1.8^{ns}± 0.1A	5.48*± 0.1B	7.2^{ns}± 0.4A
50:50	NAM	39.6 ± 2.4*C*	4.8 ± 0.2*B*	13.1 ± 0.9*C*	1.99 ± 0.0*A*	9.6 ± 0.4*B*
	AM	38.1^{ns}± 2.4B	4.5^{ns}± 0.3B	18.2^{ns}± 5.2B	2.15*± 0.0A	9.7^{ns}± 0.5B
5:95	NAM	38.2 ± 1.3*B*	4.3 ± 0.5*B*	10.2 ± 0.9*B*	2.18 ± 0.0*B*	9.4 ± 1.2*AB*
	AM	39.1^{ns}± 0.7B	5.0^{ns}± 0.2B	9.8^{ns}± 1.2AB	2.18^{ns}± 0.0A	10.9^{ns}± 0.4B
P (N)		*<0.001*	*<0.001*	*<0.001*	*<0.001*	*<0.001*
P (m)		0.615	0.343	0.463	*<0.001*	0.147
P (N x m)		0.678	0.464	0.372	*<0.001*	0.514

6.4.2.4 Nutrient concentration

The shoot S concentration (Tab. 14) was measured in all replicates and therefore a statistically analysis could be done for all three ammonium:nitrate ratios. Mycorrhizal colonization increased the shoot S concentration at the ammonium:nitrate ratios of 95:5 and 50:50. The shoot S concentration of the NAM plants was highest at the ammonium:nitrate ratio 95:5, followed by the ammonium:nitrate ratio 5:95.

At the ammonium:nitrate ratio 95:5 very little shoot material had been produced. Therefore only the total N, P, K, Mg, and NO_3^- concentrations were determined in a combined shoot sample of all four replicates (means of AM and NAM values are mentioned in the text).

Taken all values the AM treatment significantly decreased N, K, and NO_3^- concentrations in both ammonium:nitrate ratios 50:50 and 5:95. But a singular comparison revealed only a decrease of the NO_3^- concentration at the ammonium:nitrate ratio 95:5 and for the K concentration at the ammonium:nitrate ratio 50:50 (Tab. 15). The P and Mg concentrations were not significantly affected, but showed interactions between AM treatment and N speciation. Other singular comparisons revealed no differences, other than the increased P concentrations at a ammonium:nitrate ratio 5:95 in the AM plant shoots.

The N speciation treatment influenced the N concentration, but the differences in the comparison of singular treatments were not significant. The NO_3^- concentration increased significantly at the ammonium:nitrate ratio of 5:95. The part of NO_3^- in the N concentration was 1 % at the ammonium:nitrate ratio of 95:5, and 0.7 % at the ratio of 50:50, but increased to 6 % at the ratio of 5:95.

The N, P, and K concentrations of the combined samples at the ammonium:nitrate ratio of 95:5 were higher than at the other two ratios (Titel Tab. 15). The NO_3^- concentration did not differ much from those at the ammonium:nitrate ratio of 50:50. Mg concentrations were slightly lower at 95:5 than at the other two ratios.

The N, P, and Mg contents of were not significantly different (data not shown). AM treatment had no influence on the K, NO_3^- and S content of the shoots (Tab. 14 and Tab. 15). The S content decreased at the ammonium:nitrate ratio of 95:5 (Tab. 14).The K and NO_3^- content was increased significantly at the ratio of 5:95. In case of K and NO_3^- the values of the combined samples were much lower at the ammonium:nitrate ratio 95:5 than at the other two ratios (Titel Tab. 15).

Tab. 15: Effect of N speciation (N) and AM treatment (m) on shoot N, P, Mg, NO_3^-, K concentrations and total shoot K, NO_3^- content 13 weeks after planting of *Allium tuberosum*. Plants were supplied with two different ammonium:nitrate ratios and either inoculated (AM) or non-inoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Differences within the factor 'm' are denoted as significant (*) or non-significant (^{ns}) and differences between the factor 'N' are denoted with capitalized letters either *italicized* (NAM) or non-italicized (AM) as determined by the T-test (P<0.05). Values are means of 4 observations and SE (±). At the ammonium:nitrate ratio 95:5 the concentrations were for N 48.8 mg kg^{-1}, P 8.5 mg kg^{-1}, NO_3^- 0.40 mg kg^{-1} , Mg 4.3 mg kg^{-1} , and K 46.3 mg kg^{-1}, and total contents for K 64 g pot^{-1} and for NO_3^- 0.5 g pot^{-1}).

		Element concentration					Element content	
		g [kg DW]$^{-1}$					mg pot^{-1}	
NH_4:NO_3	Inoculum	N	P	Mg	NO_3^-	K	NO_3^-	K
50:50	NAM	43.0 ± 1.1*A*	3.7 ± 0.1*A*	4.6 ± 0.5*A*	0.4 ± 0.0*A*	22.9 ± 2.8*A*	3.6 ± 0.2*A*	220 ± 14*A*
	AM	41.3^{ns}± 0.7A	3.6^{ns}± 0.1A	6.0^{ns}± 0.5A	0.3^{ns}± 0.0A	14.9* ± 1.7A	2.9^{ns}± 0.2A	144^{ns}± 9A
5:95	NAM	41.2 ± 1.0*A*	3.6 ± 0.1*A*	5.8 ± 0.6*A*	2.6 ± 0.1*B*	42.7 ± 2.8*B*	24.1 ± 2.6*B*	397 ± 43*B*
	AM	38.5^{ns}± 1.0A	4.0* ± 0.1B	4.7^{ns}± 0.1A	2.1* ± 0.2B	41.8^{ns}± 2.6B	22.9^{ns}± 2.6B	455^{ns}± 18B
P (N)		*0.0347*	0.0561	0.9788	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*
P (m)		*0.0405*	0.1470	0.8109	*0.0252*	*0.0040*	0.6062	0.7394
P (N x m)		0.6123	*0.0119*	*0.0197*	0.0833	*0.0162*	0.8909	*0.0202*

6.4.2.5 Pyruvic acid and sugar

The AM treatment decreased the pyruvic acid concentration slightly (Tab. 16), but the differences remained unnoticeable in a singular comparison of the treatments. At the ammonium:nitrate ratio of 50:50, the pyruvic acid concentration was higher compared with the 5:95 ratio. The combined sample at the ammonium:nitrate ratio 95:5 showed even higher pyruvic acid concentrations (Titel Tab. 16).

Tab. 16: Effect of N speciation (N) and AM treatment (myc) on shoot pyruvic acid (PA), sucrose, fructose and glucose concentrations and total PA content 13 weeks after planting of *Allium tuberosum*. Plants were supplied with two different ammonium:nitrate ratios and either inoculated with AM (AM) or non-inoculated (NAM). Effects of the treatments were tested with a two-way ANOVA. Differences within the factor 'myc' are denoted as significant (*) or non-significant (^{ns}) and differences between the factor 'N' are denoted with capitalized letters either *italicized* (NAM) or non-italicized (AM) as determined by the T-test (P<0.05). Values are means of 4 observations and SE (±). At the ammonium:nitrate ratio 95:5 the concentrations were for PA at 4.54 µmol g FW^{-1}, sucrose at 6.2 g kg FW^{-1}, glucose at 2.3 g kg FW^{-1}, fructose at 3.2 g kg FW^{-1}.

		Concentration				Content
		µM [g FW]$^{-1}$	g [kg FW]$^{-1}$			µmol [pot FW]$^{-1}$
NH_4:NO_3	Inoc	PA	Sucrose	Glucose	Fructose	PA
50:50	NAM	3.93 ± 0.13*B*	6.6 ± 1.4*A*	4.7 ± 0.5*B*	7.85 ± 0.5*B*	155 ± 8*B*
	AM	3.63^{ns}± 0.09B	7.0^{ns}± 0.2A	5.4^{ns}± 0.1B	8.19^{ns}± 0.2B	137*± 6B
5:95	NAM	3.11 ± 0.09*A*	8.5 ± 0.5*A*	3.5 ± 0.2*A*	4.47 ± 0.2*A*	119 ± 3*A*
	AM	2.91^{ns}± 0.09A	9.5^{ns}± 0.8B	4.0^{ns}± 0.3A	4.87^{ns}± 0.3A	114^{ns}± 2A
P (N)		*<0.001*	*0.023*	*0.001*	*<0.001*	*<0.001*
P (m)		*0.030*	0.421	0.112	0.275	0.052
P (N x m)		0.654	0.702	0.801	0.924	0.257

The pyruvic acid content was not influenced by AM treatment, but the singular comparison revealed a decrease at the ammonium:nitrate ratio of 50:50. Like the pyruvic acid concentrations the pyruvic acid content also reached maximum at the ammonium:nitrate ratio of 50:50.

The sugar concentrations were not influenced by the AM treatment (Tab. 16). The sucrose concentration increased significantly at the ammonium:nitrate ratio of 5:95 compared to the ratio of 50:50. Whereas the concentrations of fructose and glucose were significantly increased at a ratio of 50:50 compared to the ratio of 5:95. The sum

of sucrose, fructose and glucose concentrations were significantly higher on the ammonium:nitrate ratio of 50:50 compared to the ratio of 5:95 (data not shown).

The combined samples at the ammonium:nitrate ratio of 95:5 showed on average slightly lower sugar concentrations than those at the other two ratios (Titel Tab. 16)

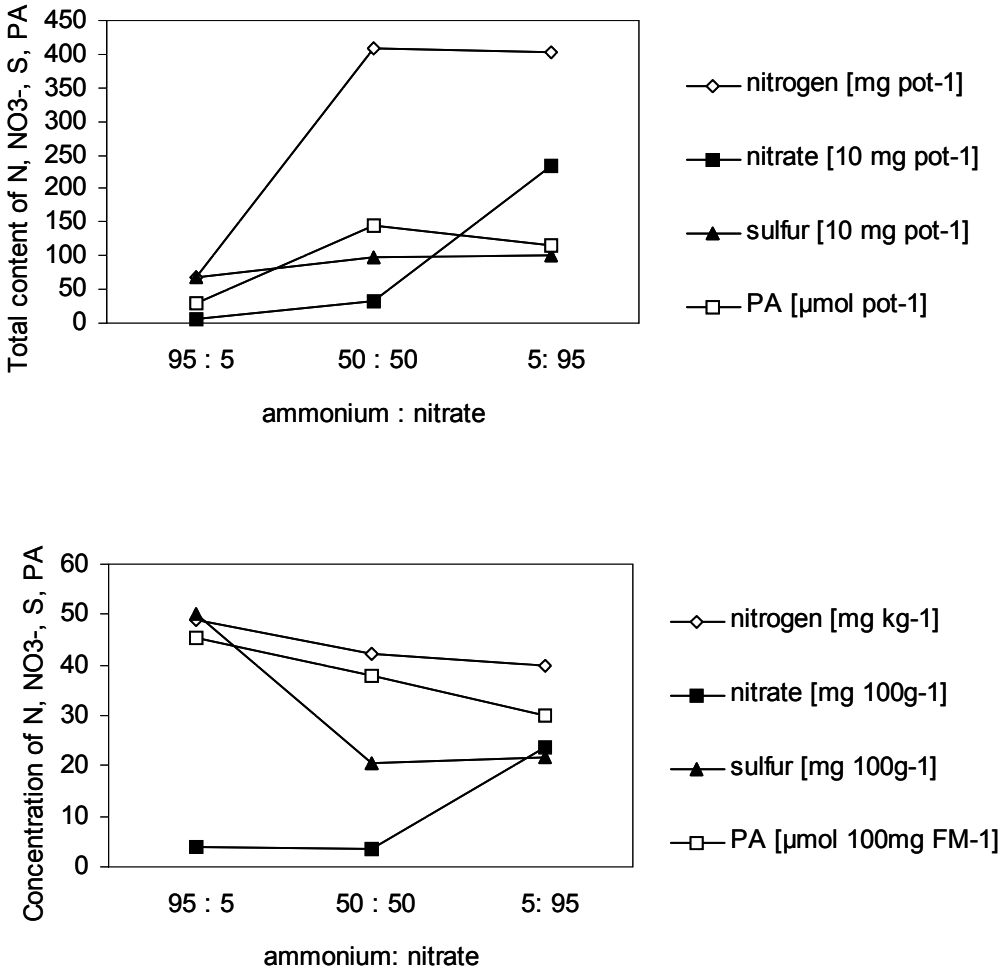


Fig. 10: Comparison of the shoot N, NO_3^-, S and pyruvic acid (PA) concentration and content at three different ammonium:nitrate ratios 13 weeks after planting of *Allium tuberosum*. Each dot combines the values of mycorrhizal and non-mycorrhizal plants.

6.5 Discussion

6.5.1 Experiment 1

The pale green young leaves and slightly darker colored older leaves at no S are a symptom of S-deficiency (Hell, 97). They grew slowly and therefore gained a very low dry weight. Plants grown at 0.2 mM S and 2 mM S supplies were only slightly different

in color and equal in their dry weight. Consequently, plants supplied with 0.2 mM S are sufficiently provided with S. A comparison with shoot element concentrations of commercially grown Chinese chive (Wills et al., 84; Rubatzky and Yamaguchi, 97; FAO, 72) reveals that shoot N and P concentrations have been satisfactory.

The shoot N and P concentrations of the 0 mM S supply are higher than at the other two S supplies, due to the slow growth. Therefore it may be assumed that also S has been accumulated. A significant decrease in P concentration was observed and also a descending tendency of N concentration at 2 mM S. Normally there is no direct competition between PO_4^{2-} and SO_4^{2-}, but high concentrations of one specific anion can sometimes suppress the uptake of other anions (Marschner, 95). The shoot S concentration at 2 mM S supply showed that even when plants grow satisfactory at a medium S supply, they are still able to accumulate higher S concentration in their tissue.

For analytical purposes the plants were left growing for almost a year with regular harvests. On 12 May 05 the plants at the 0.0 mM S supply were still growing and contained shoot S concentrations in a similar range (2.2 g kg^{-1}). This leads to the hypothesis that S^{2-} has been effectively taken up from the atmosphere through the stomata, as Durenkamp and De Kok (02; 04) found in their study: A contribution of S from atmospheric H_2S of app. 1.28 g kg^{-1} or atmospheric SO_2 was measured in the shoot after seven days of growth.

As expected the pyruvic acid concentration increases with increasing S supply. This confirms the findings of Randle and his group for garlic and onions (Randle, 92a; Randle and Bussard, 93). The soluble solid compounds are usually used to control the degree of maturation and quality in fruits and vegetables (Ahlers, 01; OECD, 02). It is an approximate value of the sugar concentration of the juice. The carbohydrate concentration of *Allium schoenophrasum,* a plant that has a similar growing pattern, is approximately 4% (FAO, 72). In comparison to *Allium schoenophrasum* the content of soluble solid compounds of *Allium tuberosum* was higher. A change of the plant composition could not be found under the influence of increasing S supply.

6.5.2 Experiment 2

6.5.2.1 Colonization

The roots of the plants grown at the ammonium:nitrate ratios of 50:50 and 5:95 were sufficiently colonized, but the root length colonization lowered with rising NH_4^+ supply in the nutrient solution. One reason could be the correspondingly decreasing pH of the

nutrient solution. The NH_4^+ entry into cells is in counter transport with protons (Britto and Kronzucker, 05), which acidifies the rhizosphere. The buffer used in this experiment was not strong enough. We chose the concentration of the MES buffer in accordance to the literature. But most experiments were either done in pure nutrient solution systems or in steady dripping irrigation systems (Bugbee and Salisbury, 85; Miyasaka et al., 88; Hawkins et al., 00). In our experiment the nutrient solution probably formed only a thin layer around the roots during the watering process, which volume was too small to buffer the H^+ ions efficiently.

Other studies found that the colonization of roots was reduced by lower pH. The optimal pH conditions for spore germination for *Glomus spp*. is between 6 and 9 (Green et al., 76), root colonization and growth of AM fungi (Ortas and Rowell, 04).

6.5.2.2 Fresh and dry weight of shoots and roots

AM fungi had no significant influence on the growth of the plants (Tab. 14). The effect of AM fungi base on the ability of its hyphae to explore soil aggregates for some nutrients, particularly P, which are not available to the roots on deficient soils. In our nutrient solution all plant relevant macro- and micronutrients for a sufficient growth were provided. Bago and Azcón-Aguilar (97) found that in case of NH_4^+ supplemented plants, a strong stimulation in growth was induced by the establishment of the symbiosis. The expected support by AM treatment for growth in the presence of high NH_4^+ supply was not reproduced in this experiment. Hawkins and George (01) also found no difference in growth between AM and NAM plants, but their NO_3^- fed plants were larger than their NH_4^+ fed plants. In this experiment the Chinese chive shoot habitus and weight did not differ significantly between the ammonium:nitrate ratios of 50:50 and 5:95. Although the roots at the ratio of 50:50 showed the highest dry weight for AM plants, the variation was too large to draw firm conclusion.

At the highest NH_4^+ treatment the growth of the plants was retarded and the older leaves wilted from the tip. The cause could be an increase of chloride (Cl) uptake in presence of high NH_4^+ concentrations in the substrate, resulting in salinity stress (Britto et al., 04).

High concentrations of NH_4^+ can be toxic to some species and can therefore be responsible for the changes (Miller and Cramer, 05). Whereas NO_3^- is stored in vacuoles without detrimental effect, NH_4^+ and in particular its equilibrium partner ammonia (NH_3) are toxic at quite low concentrations. The NH_4^+ concentrations are usually lower than 15µM in the cytoplasm. The main pathway of detoxification within the plant is the formation of amino acids, amides and related compounds. To compensate the change

in charge, protons are released into the rhizosphere decreasing the pH. This may affect the roots growth and eventually cause leaves to wilt (Marschner, 95; Claussen and Lenz, 95). Date et al. (05) found that addition of Cl at 8.5×10^{-3} mM with 0.67 mM NH_4^+ to a nutrient solution caused wilting of lettuce leaf tips, because of the formation of chloramines in the solution. NH_4^+ without Cl did not show any wilting or browning of roots. Long experience showed that a nutrient solutions containing Cl up to 5 mM had no toxic chlorine effect on most plants (V. Römheld, personal communication). The present experiment had Cl concentrations of 4, 11 and 16 mM, found decreasing pH with rising NH_4^+ supply, and found as well wilting of leaves only within the highest ammonium:nitrate ratio, matching the findings of Marschner, Date and Römheld.

Another reason for the decreased growth with increased NH_4^+ supply could be a NO_3^- deficiency (2mM sole NH_4^+ supply) (Walch-Liu et al., 00). Ammonium in the external solution strongly suppresses net uptake of NO_3^-. Rapid NH_4 influx into cytoplasma and a decrease in transmembrane potential are supposed to be possibly involved in the rapid suppression of net NO_3^- influx (H^+-NO_3^- symport) (Marschner, 95). These assumptions are supported by our observations during alteration of the NO_3^- concentrations.

Other growth depressions by application of NH_4^+ as a sole nitrogen source, such as uncoupling of photophosphorylation, lack of carbohydrates or nutrients, and impairment of water status (Walch-Liu et al., 00) are unlikely, because the results showed that our plants had normal sugar and element concentrations and had no drought stress.

6.5.2.3 Element concentration and content

The element concentrations in the plant leaves of this experiment were compared with those of commercially grown Chinese chive (Wills et al., 84; Rubatzky and Yamaguchi, 97; FAO, 72). Shoot N and P concentrations were in the same range, whereas the shoot Mg concentrations were even higher in our experiments. The shoot K concentration at an ammonium:nitrate ratio of 5:95 was equally high, but at the ratio of 50:50 only half in our case. A comparison with our first experiment showed, that the shoot S concentration was in a lower range (Tab. 13). The medium S supply was chosen in experiment 2 to bring out the effects from the treatments more clearly.

The high shoot S concentration and low shoot S content at the ammonium:nitrate ratio of 95:5 were a result of S accumulation due to slow growth (Tab. 14). In relationship to the shoot N concentration (Fig. 10) these shoot S concentration was increased

strongly from the ratio of 50:50 to 95:5. These findings reveal that it is possible that the high ammonium:nitrate ratio supported the SO_4^{2-} uptake, whereas the medium ratio of 50:50 shows not increase in shoot S concentration or contents compared to the low ratio of 5:95 (Tab. 14).

The increased K concentration and total K content at the ammonium:nitrate ratio of 5:95 compared to 50:50 may be explained by an increased uptake via channels and transporters induced by NO_3^- (Van Beusichem et al., 88; Wang et al., 01).

The NO_3^- concentrations at the ammonium:nitrate ratios of 95:5 and 50:50 are almost similarly low. In contrary the NO_3^- concentration and content at the ammonium:nitrate ratio of 5:95 are high. The reason might the translocation of NO_3^- from the roots to the leaves for storage and to accelerate the NO_3^- reduction. This has been observed at high NO_3^- supply and high light intensity (Britto and Kronzucker, 05; Köhler et al., 02). The other nutrients N, P, and K concentrations were only increased at an ammonium:nitrate ratio of 95:5 due to slow growth, whereas Mg was decreased in competition with NH_4^+.

Mycorrhizal colonization did not contribute to the uptake of N (George, 00). Interestingly the proportion of NO_3^- from total nitrogen is lower in AM colonized plants (3%) than in NAM plants (3.5%). This is probably due to the findings of Hawkins (Hawkins et al., 00; Hawkins and George, 01) that AM hyphae favorably use NH_4^+ as N source. This acceptation is assisted by the significantly increased K concentration in NAM plants, the counterion of NO_3^- in uptake.

As expected, the shoot P concentration was not enhanced by the AM. Probably the P contribution through the hyphae became redundant, because of the high offer of P in the nutrient solution and uptake through the roots.

The shoot S concentration was increased by AM at the ammonium:nitrate ratios 95:5 and 50:50, whereas the N and NO_3^- concentrations were decreased (Tab. 14 and Tab. 15). The S and N metabolisms are closely coupled. Sulfur uptake and assimilation has been shown to be dependent upon N availability. The data of Wang et al. (00; 03) suggests that NO_3^- can induce genes of SO_4^{2-} uptake and assimilation and, thereby, may increase SO_4^{2-} assimilation rates or capacity. This may be true for the highest NO_3^- supply where S concentration was increased in the NAM plant. But in the treatments of the present experiment an influence of NO_3^- on the uptake of S was poor when NH_4^+ supply was increased in association with AM fungi. This corresponds with observations that NH_4^+ supply increases the uptake of SO_4^{2-} and incorporation into proteins (Brunold and Suter, 84; Koprivova et al., 00). It was assumed (see above) that the mycorrhizal

plants took up preferentially NH_4^+. As a result the plants might have taken up more SO_4^{2-} to balance the ions.

6.5.2.4 Pyruvic acid

The pyruvic acid concentration did not correlate with the shoot S concentrations as expected. In contrary to sulfur AM decreased pyruvic acid concentration and content. A comparison of the shoot pyruvic acid, N, S, and NO_3^- concentrations (Fig. 10) shows that the higher pyruvic acid concentrations were induced by the higher N concentrations at the ammonium:nitrate ratios of 95:5 and 50:50, but that with higher rates of NO_3^- supply the formation of pyruvic acid decreased. The comparison of the N, S, NO_3^-, and pyruvic acid content (Fig. 10) reveals similar results. Shoot S content did not correlate with the shoot pyruvic acid content. In contrary the shoot pyruvic acid content rose with the shoot N content at the ammonium:nitrate ratio of 95:5 to 50:50, but decreased with an increased NO_3^- content in the shoot. The highest formation of pyruvic acid was reached at ammonium:nitrate ratio 50:50. It can therefore be added to the results of Coolong and Randle (03), that at low sulfur supply is pyruvic acid not only influenced by the rate of nitrogen supply, but also by the nitrogen speciation.

6.5.2.5 Sugar

A increased NH_4^+ supply at a ammonium:nitrate ratio of 50:50 compared to the ammonium:nitrate ratio of 5:95 lowered the sucrose concentrations and increased the fructose and glucose concentrations in the leaves (Tab. 16). In tomato (Bialczyk et al., 05) or tobacco (Matt et al., 01) plants a comparable variation of sucrose concentration as well as of glucose and fructose on the N supply at an ammonium:nitrate ratio of 50:50 has not been observed. Therefore the results may be better explained by the observations of Curatti et al. (06). They observed an increased expression of genes for sucrose synthase activity with higher NH_4^+ supply compared to NO_3^- supply in cyanobacteria. Cyanobacteria and higher plants have a similar set of enzymes to cleave sucrose by sucrose synthase or invertases, when there is a high demand of hexoses (Winter and Huber, 00; Salerno and Curatti, 03). Hexoses, such as glucose and fructose, are precursors of α-ketoglutarate (Heß, 99). The α-ketoglutarate serves as C skeleton for the assimilation of NH_4^+ into amino acids, such as glutamine and glutamate (Ferrario-Méry et al., 05). It is therefore suggested that the decrease of sucrose and increase of fructose and glucose at an ammonium:nitrate ratio of 50:50 results from an increased cleavage of sucrose to glucose and fructose due to activity of sucrose synthase. Additionally the glutamine synthetase activity has been observed in tobacco plants to increase during the day and remain high most of the night at an ammo-

nium:nitrate ratio of 50:50 compared to a dominant NO_3^- supply (Matt et al., 01). In this time the plant accumulates much higher levels of NH_4^+ (Matt et al., 01). These results support the idea, that sucrose is increasingly cleavaged for the assimilation of NH_4^+.

The significantly higher sum concentrations of all three sugars at an ammonium:nitrate ratio of 50:50 than of 5:95 may have also been effected by the higher requirement of readily available C skeletons, whereas NO_3^- could have been stored in vacuoles for delayed assimilation.

In conclusion these experiments indicated that (a) 0.2 mM S in the nutrient solution was sufficient for growth of *Allium tuberosum*, (b) increasing S concentration in the substrate and in the shoot corresponded with pyruvic acid concentration, (c) AM fungi increased shoot S concentrations at high NH4+ supply, but not the shoot dry weight or pyruvic acid concentrations, and (d) the supply of an ammonium:nitrate ratio of 50:50 seemed to be the preferential N form for *A. tuberosum* to produce plants with high yield of health related organosulfur compounds. This was due to a similar dry weight as with the dominant NO3- supply and to higher total content of pyruvic acid. *A. tuberosum* is a well suited experimental plant, because of its uniform growth and possibility for multiple harvests.

Chapter 7

7 General Discussion

The previous chapters of this thesis describe results obtained in several separate experiments. In this last chapter, the main results of these experiments are discussed in view of the hypotheses given in the General Introduction. Finally, some perspectives for further research are presented.

7.1 Effect of substrate and nitrogen speciation on the extent of arbuscular mycorrhizal colonization

Peat, peat-compost and perlite substrates were used in some experiments as examples of substrates commonly used in horticulture. These substrates were tested for their suitability for colonization by AM fungi of roots of a variety of plant species.

In general, plants were colonized successfully on inoculated commercial peat substrates, commercial peat-compost substrates and specifically prepared peat-compost substrates. The non-inoculated peat and peat-compost substrates were apparently free of any infectious AM propagules.

Root length colonization was decreased on peat substrates with increasing compost amendment rates in several experiments. This decrease in root length colonization on peat substrates with 40% compost amendment compared to a 20% amendment was found in leek (Chapter 1), in pelargonium, and poinsettia plants (data not shown). This decrease in colonization is probably linked to a higher availability with higher addition of compost. When nutrient supply is abundant, AM colonized plants are less dependent on the fungus (Koide and Mosse, 04; Lerat et al., 03). Higher nutrient supply to a substrate can suppress fungal growth (Vierheilig, 04; Pinior et al., 99). On metabolic reason for the suppressive effect of high nutrient supply on AM colonization may be a partial C immobilization in the plant, because high P and N availability to the plant may reduce C flow to AM fungal structures (Olsson et al., 05b). Another explanation for this result is that the higher water holding capacity of the 40% compost substrate compared to the 20% compost substrate. Substrate water supply can affect mycorrhizal colonization. Some AM fungal species show a decreased hyphal growth in moist soils (Smith and Read, 97).

An exception to the results described above was observed for pelargonium plants in chapter 3, experiment 1, where root length colonization on peat-compost substrate was not significantly affected by the rate of compost additions. Probably, the nutrient de-

mand of the pelargonium plants was probably not completely met by either compost level.

For plants grown on perlite, colonization was observed, just as for the other substrates, only in the inoculated treatment. As expected, the non-inoculated perlite remained free of living mycorrhizal fungi. This result can be explained by the perlite production process, in which the temperature exceeds 1000°C (information from the supplier: Knauf Perlite GmbH, Dortmund, Germany). Moreover, before the experiments started the substrate was autoclaved.

Experiments with different ammonium:nitrate ratios on perlite showed a decrease in root length colonization with increasing ammonium:nitrate ratio. Roots of different *Allium* species, such as *A. tuberosum* (Chapter 5, Expt. 2), *A. fistulosum, A. sativum,* and *A. cepa* (data not shown), had root length colonization rates of up to 80% at a low ammonium:nitrate ratio. Colonization rates decreased strongly with increasing ammonium:nitrate ratio. This effect may be explained by the decreasing pH in the root environment. The pH in the rhizosphere drops due to dominant NH_4^+ uptake. As a result, AM spore germination, hyphal growth, and root colonization are suppressed (Green et al., 76; Ortas and Rowell, 04). Moreover, high NH_4^+ supply can also directly reduce root and/or extraradical hyphal biomass (Hawkins and George, 01; Olsson et al., 05a).

The exceptional low root length colonization of *A. fistulosum* on all ammonium:nitrate ratios described in chapter 4 may be explained by the sufficient nutrient supply in this experiment. In conditions of nutrient sufficiency plants down-regulate AM colonization and hyphal growth (Vierheilig, 04; Pinior et al., 99).

7.2 Effect of arbuscular mycorrhizal colonization on shoot dry weight and nitrogen, phosphorus, potassium, sulfur and zinc and uptake

The often observed positive effect of AM colonization on plant growth is based on the fungal nutrient contribution to the plant metabolism. This fungal contribution relies on its ability to explore a larger soil volume and penetrate into smaller pore diameters than is possible by roots (Drew et al., 03). In this way, hyphae enlarge the depletion zone around roots. Moreover, the transport of nutrients via hyphae is much faster than by diffusion in soil. Also, hyphae are better at competing against free-living soil microorganisms for recently mineralized nutrients than are roots (Smith and Read, 97). These beneficial effects may become apparent on agricultural soils that are deficient in a certain nutrient. Since the fertilization management techniques used in organic agri-

culture sometimes result in low nutrient availability for plants, AM are particularly important in organic production systems.

Mycorrhizal fungi have also been reported to protect plants from other forms of stress. Stress in plants induced by high NH_4^+ fertilization is called NH_4^+ toxicity (Van den Berg et al., 05; Britto and Kronzucker, 02; Lucassen et al., 03). For microorganisms, however, NH_4^+ is the most important source of mineral N (Stitt et al., 02). Consequently, it is likely that NH_4^+ is less toxic to AM-colonized plants than to non-colonized plants. Therefore, it was hypothesized that AM would increase shoot nutrient concentrations and shoot dry weight on peat substrates with low P availability even at higher compost amendment rates, and on perlite with a higher ammonium:nitrate ratio.

For peat and peat-compost substrates, however, a beneficial AM effect on shoot dry weight was not generally apparent (Chapters 1-3). Moreover, shoot P concentration often was not increased (Chapters 1 & 2). Most evidence of increased P uptake in mycorrhizal plants comes from experiments and observations on mineral soils. Also, freshly applied organic P sources can be utilized by AM fungi (Feng et al., 03). However, the present experiments gave evidence that plant P uptake from organic substrates such as peat or compost is less dependent on AM fungus colonization than is P uptake from soils with mineral P sources. Compost may contain P sources that are either readily accessible to plants or inaccessible to plants and AM fungi alike. In the latter case, the P inaccessibility might be due to physico-chemical fixation of P in form of condensed calcium phosphates such as apatites or octacalcium phosphates (Frossard et al., 02; Grey and Henry, 99).

In contrast to the above mentioned observations, P uptake in pelargonium plants was increased by AM inoculation in experiment 1 of chapter 3. It can be assumed that the shoot P concentration in young plants was low enough to induce an uptake of P by AM.

The present data show for the first time that on peat-compost substrates Zn and K uptake was actively supported by AM colonization (Chapters 1 & 3). For Zn, this was the case when the Zn status (Chapter 1, Expt. 2) of non-mycorrhizal plants was relatively low. When the nutrient status of non-mycorrhizal plants was higher, the effect of AM fungal colonization on Zn uptake was less (Chapter 1, Expt. 1). In contrast, K uptake was also increased in mycorrhizal plants on substrates that contained sufficient K (Chapter 1, Expt. 1). Thus, mycorrhizal Zn uptake was apparently regulated by the plant Zn demand, while mycorrhizal K uptake was probably a by-process of the general metabolic activity of the fungus irrespective of plant K demand. Hyphae of AM fungi can transport not only P (George et al., 92), but also Zn and K (George, 00). This can lead to increased K and Zn concentrations in mycorrhizal plants. The contribution of K

to plants by AM fungi has previously been described only for acidic soils (Clark and Zeto, 00; Alloush and Clark, 01) and in one report for a peat substrate at pH 5.8 (Nowak, 04). It can be speculated that the decomposition of the organic compost material released pH reducing humic acids which increase K availability in the substrate. It may even be possible that small aggregates of compost and peat remained acidic in the limed environment, and that hyphae were able to enter and exploit those acidic aggregates.

On perlite, an AM induced increase in shoot dry weight in plants fertilized with a high ammonium:nitrate ratio could be realized for *A. fistulosum* (Chapter 4), *A. sativum* (data not shown), and *A. tuberosum* (data not shown). This effect did not occure with higher rates of nitrate supply. No supporting effect of AM was found on uptake of N, P, K, Zn, Mg or Cu in *A. tuberosum* and *A. fistulosum* grown on perlite (Chapters 4 & 5). These results indicate that the plants on perlite had been sufficiently supplied with those nutrients. In addition, bare roots systems may be ideally suited to use percolating nutrient solution, so that hyphal element uptake may be neglible under conditions of perlite-nutrient solution experiments. The uptake of S is separately discussed below.

For a test of the support for plants P acquisition from rock phosphate by AM fungi, the fast-growing lettuce plant was used (Chapter 2). It was hypothesized that AM colonization would increase P mineralization from rock phosphate on peat substrates. Arbuscular mycorrhizal fungi, however, did not increase P uptake from peat substrates amended with rock phosphate. Moreover, AM also did not increase shoot dry weight. Apparently, the P mobilization mechanism of AM did not increase the availability to lettuce of P from rock phosphate. These results can be interpreted in two ways: Firstly, the finely branched root architecture of lettuce plants and an increase in the root hair density of non-mycorrhizal plants induced by low P availability (Gahoonia and Nielsen, 98; Ma et al., 01) may have fulfilled similar functions to those provided by the hyphae of the mycorrhizal plants (Jakobsen et al., 05; Chen et al., 05). Alternativiely, calcium phosphate (apatite), the main component of rock phosphate, may have been inaccessible to plants and AM fungi alike. The latter scenario is consistent with the results that were obtained on peat compost substrates (Chapters 1 & 3), where the plant-available P fraction was probably fixed in the form of slowly soluble calcium phosphates (Frossard et al., 02). An exception to this observation of limited P mobilization by AM fungi was found only once, in pelargonium plants (Chapter 3, Expt. 1).

Organic management has been reported to increase the biodiversity of AM compared to conventionally-managed soils (Mäder et al., 02; Oehl et al., 04; 05). It was hypothesized that AM strains originating from organically managed or natural habitats would

be superior at mobilizing P from rock phosphate compared to a commercial inoculum. Overall, the AM strains did not increase the acquisition of P by lettuce plants, compared to non-inoculated plants (Chapter 2). Nevertheless, there was a tendency that the commercial AM inoculum and the isolate from an organic-dynamically managed soil were superior in mobilizing P from rock phosphate compared to the isolate from a nature conservation area.

7.3 Effect of arbuscular mycorrhiza colonization on flower development

Mycorrhizal ornamental plants have often been observed to develop and flower earlier compared to non-mycorrhizal plants, although the mechanism responsible for this phenomenon is not clear. It was hypothesized that AM would increase flower and bud development in pelargonium and poinsettia. The number of buds and flowers were in fact in the present study significantly increased by AM fungi, and shoot K concentration corresponded directly and positively with the number of buds and flowers (Chapter 3). In these experiments, shoot P and K concentrations reached levels generally regarded as sufficient for these species only in the inoculated treatments. However, the flowering effect of AM colonization can probably not be credited solely to the higher plant nutrient uptake, because the differences in shoot nutrient concentrations between mycorrhizal and non-mycorrhizal plants were not always statistically significant.

The positive effect of increased shoot K concentration on flowering was evident. Potassium performs in a wide range of functions in plants (Marschner, 95). For example, K acts as a carrier ion in xylem and phloem, transporting solutes, assimilates, and hormonal stress signals such as abscisic acid (Peuke et al., 02). Higher levels of K in a plant could be responsible for quicker transport of phytohormones, such as gibberellins, inducing bud production. The production of such hormones may also be increased by mycorrhizal colonization.

7.4 Effect of arbuscular mycorrhizal colonization and of a high ammonium:nitrate ratio on organosulfur compounds in plants

Arbuscular mycorrhizal fungi may influence secondary plant metabolism, as was shown above in the case of flower development. *Allium* species are easily colonized by AM (Fusconi et al., 05), and they contain secondary metabolites that support human

health in several ways, such as organosulfur compounds investigated in this study (Kodera et al., 03).

Previous studies have shown that the concentration of organosulfur compounds increases in *Allium* species with increasing S concentration in plant tissue and external solution (Randle, 92b; Randle and Bussard, 93), and they can be influenced by S and N fertilization (Coolong and Randle, 03). In general, ammonium (NH_4^+) absorption into roots supports the uptake of anions like sulfate (SO_4^{2-}). Nitrate (NO_3^-) uptake can suppress the uptake of other anions, a consequence of the plant's maintenance of ionic balance (Marschner, 95).

It was therefore hypothesized that increasing the ammonium:nitrate ratio in the external solution would increase the uptake of SO_4^{2-} and therefore increase the concentration and/or content of organosulfur compounds in the plant. Although high concentration of NH_4^+ in the substrate solution can be toxic to plants, NH_4^+ is the most important source of mineral N for microorganisms, such as AM (Stitt et al., 02). Thus AM fungi should support growth of mycorrhizal plants. Therefore, it was also hypothesized that, in onion plants fertilized with a high ammonium:nitrate ratio, AM colonization would increase plant growth, stimulate secondary plant metabolism, and consequently increase plant tissue content of organosulfur compounds.

In the present experiments, organosulfur compounds responded to two fertilization treatments: First, their concentration in the shoot tissue was increased by increasing S concentration in the substrate solution and in the shoot (Chapter 5, Expt. 1). Secondly, their concentration and content in the A. *tuberosum* plant was higher when N was supplied with a intermediate ammonium:nitrate ratio compared to a low one (Chapter 5, Expt. 2). These results indicate that organosulfur compounds in the plants are influenced not only by S and N supply, but also by N speciation.

The results of the present study show that increased yield of organosulfur compounds can be achieved at intermediate ammonium:nitrate ratio that also supports high shoot biomass. The increase in biomass may have been the result of either plant genuine NH_4^+ tolerance (Chapter 5, Expt. 2) or by effects AM colonization against plant NH_4^+ toxicity (Chapter 4). However, an influence of AM on shoot concentration of organosulfur compounds could not be detected (Chapters 4 & 5, Expt. 2).

Shoot S concentration in *A. tuberosum* was increased in association with AM fungi at high NH_4^+ supply (Chapter 5, Expt. 2). Perhaps, mycorrhizal plants took up NH_4^+ in preference to NO_3^-, and the lower NO_3^- uptake facilitated SO_4^{2-} uptake by those plant. Interestingly, the concentration of organosulfur compounds in the shoot did not correspond to changes in shoot S concentration when the external S concentration remained

the same (Chapter 4 & 5, Expt. 2). Presumably, the increases in shoot S concentration induced either by increasing ammonium:nitrate ratio (Chapter 5, Expt. 2) or by AM (Chapter 4) were not sufficiently distinct to increase the production of organosulfur compounds.

The most NH_4^+-tolerant *Allium* species was *A. tuberosum* (Chapter 5, Expt. 2). It produced similar dry weight at low and intermediate ammonium:nitrate ratios. However, in this species the high shoot NO_3^--concentrations that were seen in the low ammonium:nitrate treatment were associated with decreased concentration of organosulfur compounds in the plant.

Allium fistulosum (Chapter 4) and *A. cepa* (data not shown) plants preferred a low ammonium:nitrate ratio. In this situation, they had highest dry weights and organosulfur contents. In the case of mycorrhizal *A. fistulosum* plants, however, dry weight and organosulfur content were similarly high on the low and intermediate ammonium:nitrate ratios (Chapter 4). High NH_4^+ fertilization was accompanied by low pH in the medium. Recent experiments have shown that NO_3^- and NH_4^+ are assimilated into arginine at the tip of the mycorrhizal hyphae, transported to the plant, and transferred probably as NH_3 through the fungus-plant interface (Govindarajulu et al., 05). In this case, the acidification related to ammonium uptake may not take place directly in the rhizosphere, and root damage could be reduced.

At the high ammonium:nitrate ratio, plants showed reduced growth and wilting symptoms (Chapters 4 & 5). The main pathway of NH_4^+ detoxification of ammonium within the plant is the formation of amino acids, amides and related compounds. The uptake of NH_4^+ into the plant is accompanied by a release of protons into the rhizosphere, resulting in a decline in rhizosphere pH. Low pH can affect root growth and, as a consequence, cause leaf wilting (Marschner, 95; Claussen and Lenz, 95). It is documented (Date et al., 05) that addition of chlorid (Cl) at 8.5×10^{-3} mM with 0.67 mM NH_4^+ to a nutrient solution caused wilting of lettuce leaf tips, because of the formation of chloramines in the solution. Leaf wilting in the present study occurred only on nutrient solutions with the highest NH_4^+ concentration (16 – 26 mM Cl).

7.5 Perspectives for further research

This thesis covered several aspects of the influence of AM on plant growth and composition. New questions arose during the study that should be investigated in more detail in further experiments. Some of these points are summarized in the following:

From results presented in chapters 1 to 3, it may be assumed that peat and peat compost substrates lack beneficial microorganisms like AM or bacteria. In contrast, in soils many AM symbioses live in tripartite associations with bacteria (Bonfante, 03; Rillig et al., 05; Barea et al., 05; Toljander et al., 06). It is therefore likely that the availability of P from rock phosphate or N from horn meal can be increased by incorporating AM together with P-solubilizing and N-solubilizing bacteria into the substrate. An efficient combination of mycorrhizal fungi and P-solubilizing bacteria has been reported to help in plants rock phosphate utilization (Barea et al., 75; 02).

Also interesting is the further investigation of flower development of mycorrhized plants. Here, the measurement of phytohormones like auxin and gibberellin in combination with PO_4^{3-}, K, and Zn fertilization should improve the understanding of flower development. This topic may contain economic potential for ornamental plants in horticulture.

To maximize the content of health related compounds of the different *Allium* species, it is important to test each *Allium* species separately for its optimal ammonium:nitrate ratio. Moreover, metabolic mechanisms other than nutrient uptake should be considered when attempting to increase the content of organosulfur compounds. The key activities of these compounds include reproduction, defence, pathogenicity, stress resistance and resource storage in plants (Jones et al., 04). Further investigation concerning the mechanisms of these key activities might lead to increased production of organosulfur compounds in commercial production of *Allium* species.

Moreover, *Allium* species also contain health-related compounds other than organosulfur compounds. Phenols are antioxidants that could also be tested for their response to different fertilization regimes.

Another important factor for the consumer besides the nutrient value of edible plants is their taste. Changes in N speciation supply may also alter the pungency, sweetness, and juiciness of *Allium* species, and these changes would need to be evaluated as well.

8 Summary

Arbuscular mycorrhizal (AM) fungi can be beneficial for horticultural crops due to their nutrient acquisition properties and stimulation of the plant metabolism. These characteristics of AM fungi may be of relevance for two topics that recently became a focus of both producers and consumers: organic agriculture and plant quality (secondary metabolite production) in crops. These topics with high practical relevance have until now only been sparsely investigated.

The present work investigates the prospects of AM fungi in horticultural production and thus engages in the rarely investigated interface of practice and research. It focuses on the prospects of AM fungi a) to solve plant nutritional problems, b) to induce the flower development of ornamental plants, and c) to improve the health potential of crop plants for humans.

Plant nutritional problems and the contribution of AM fungi were investigated with leek, pelargonium and poinsettia plants on peat-based substrates with 20% and 40% compost additions (chapter 1 & 3), lettuce plants on peat-based substrates grown with substrate own P, and with additions of rock phosphate, or highly soluble P (chapter 2), and bunching onion and chinese chive fertilized with a nutrient solution with low, medium and high ammonium:nitrate ratios in perlite (chapter 4 & 5). Mycorrhizal colonization, dry weight, and N, P, K, S, NO_3^-, Mg and Zn concentrations in plants were measured.

On peat based substrates, inoculation with AM fungi resulted in colonization rates of up to 70% of total root length in leek (chapter 1), 36% in pelargonium and 2% in poinsettia (chapter 3). Mycorrhizal fungus colonization increased shoot Zn and K concentrations in leek, shoot P and K concentrations in pelargonium, but did not significantly affect shoot dry matter or shoot N concentrations. Similarly AM colonization (up to 65%) of lettuce plants (chapter 2) did not promote dry matter production on substrate own P and rock phosphate (low P availability), or highly soluble P (high P availability) compared to the non mycorrhizal plants. Although AM colonization increased shoot N, Mg, and Zn concentrations in lettuce plants, shoot P concentration was not increased (chapter 2). Compost addition to peat-based substrates (chapter 1 & 3) increased plant growth, shoot P concentration and in leek also shoot K concentration, but addition of compost did not always completely meet plant nutrient demand. Phosphorus seemed to be fixed on peat based substrates and therefore less available to AM fungi compared to soils, whereas K was especially available on peat based compost substrates.

On perlite, AM colonization rates of *Allium fistulosum* (chapter 4) were low, but AM colonization drastically increased dry matter production of plants at high and medium ammonium:nitrate ratios (low pH). The AM colonization had no impact on the growth

of *A. tuberosum* (chapter 5). Here, highest colonization rates (43%) were observed at a low ammonium:nitrate ratio, decreasing with increasing rate of ammonium supply. Generally mycorrhizal colonization did not significantly increase shoot N, S, and P concentrations of *A. fistulosum* or shoot N, P, K, Mg and NO_3^- concentrations in *A. tuberosum*. Only shoot NO_3^- concentrations in *A. fistulosum* and shoot S concentrations in *A. tuberosum* were increased by AM colonization. The highest rate of ammonium supply increased shoot N and P concentrations in *A. fistulosum*. Dry matter production of *A. fistulosum* increased with the supply of N at a low ammonium:nitrate ratio compared to the other two ratios of N supply. In contrast, *A. tuberosum* had similar dry matter production on low and medium ammonium:nitrate ratio. An effect of AM colonization on nutrient uptake was rare due to the high nutrient supply. The benefit of AM fungi against ammonium toxicity could be sparsely observed.

Mycorrhizal effects on bud and flower development were investigated by inoculating pelargonium and poinsettia plants with three commercial AM inocula in combination with 20% and 40% compost additions on peat-based substrates (chapter 3). Mycorrhizal colonization increased the number of buds and flowers in both plant species.

Treatment effects on secondary metabolites in *A. fistulosum* and *A. tuberosum* were determined by exposing mycorrhizal and non mycorrhizal plants to three ammonium:nitrate supply ratios. The compounds measured were singular sugars (glucose, fructose, and sucrose), total soluble solids, and organosulfur compounds (measured as pyruvic acid). In *A. fistulosum* (chapter 4) the ammonium:nitrate ratio and AM colonization, and in *A. tuberosum* (chapter 5) AM colonization had little effect on singular sugar, soluble solid compounds, or pyruvic acid concentration. However, *A. tuberosum* grown at a medium ammonium:nitrate ratio had increased pyruvic acid concentrations compared to plants grown at a low ammonium:nitrate ratio. Non-mycorrhizal *A. fistulosum* plants performed best in terms of shoot growth and quality when supplied with N at a low ammonium:nitrate ratio. However, when *A. fistulosum* was protected by AM colonization against inhibiting effects of high ammonium supply, the plants produced similar amounts of organosulfur compounds at a low and medium ammonium:nitrate ratios. In contrast, for *A. tuberosum* a medium ammonium:nitrate ratio was preferential for the production of high amounts of health related organosulfur compounds irrespectively of AM colonization.

All non-mycorrhizal treatments remained free of AM colonization in all used substrates, although the peat-based substrates and compost were not sterilized before use. On the other hand high rates of AM colonization could be obtained after AM inoculation. This observation that all substrates used in this study did not support spontaneous mycorrhizal colonization is of high practical significance. Horticultural producers must

use inoculation and relatively low nutrient addition rates if they intend to grow mycorrhizal plants on these substrates.

Colonization improved plant nutrient status and flower development. Under the described experimental conditions, however, plants did not consistently benefit in growth or plant composition from the mycorrhizal symbiosis. Additions of compost were a means of improving the substrate quality for an increased plant nutrient acquisition and plant growth in organic horticulture. The plant quality of *Allium* species in respect to organosulfur compounds was increased by taking the individual *Allium* species into consideration, their specific requirements for an optimal ammonium:nitrate supply ratio, and a possible AM effect on plant growth.

9 Zusammenfassung

Aufgrund seines Nährstoffaneignungsvermögens und seiner Stimulierung des Pflanzenmetabolismus kann der Arbuskuläre Mykorrhiza (AM) Pilz im Gartenbau nutzbringend eingesetzt werden. Diese Eigenschaften des AM Pilzes könnte für zwei Themen an Bedeutung gewinnen, die in den letzten Jahren in den Blick von Produzenten und Konsumenten rückten: den ökologische Landbau und die Pflanzenqualität (Primäre und Sekundäre Pflanzenstoffe). Diese Themen haben eine hohe praktische Relevanz, wurden aber bisher in dieser Hinsicht nur wenig wissenschaftlich untersucht.

Die vorliegende Arbeit beschäftigt sich mit den Möglichkeiten von AM Pilzen im Gartenbau und betrachtet damit die nur selten untersuchte Schnittstelle zwischen Praxis und Forschung. Die Schwerpunkte der Arbeit liegen auf der Möglichkeit von AM Pilzen a) pflanzenernährerische Probleme zu lösen, b) die Bildung von Blüten bei Zierpflanzen zu induzieren und c) das Gesundheitspotential von Gemüsepflanzen für den Menschen zu erhöhen.

Der Beitrag von AM Pilzen zu der Lösung von pflanzenernährerischen Problemen wurde anhand von Porree, Pelargonie und Poinsettie auf einem Torf-basiertem Substrat mit 20% und 40% Kompostzusatz (Kapitel 1 & 3) untersucht. Des Weiteren wurde Salat auf Torf basierten Substraten mit unterschiedlichen Phosphorbehandlungen getested: substrateigenem P und Zugabe von Rohphosphat und gut löslichem P (Kapitel 2). Zuletzt wurden Chinesische Frühlingszwiebeln und chinesischer Schnittknoblauch mit Nährlösungen auf Perlit ernährt, die jeweils ein niedriges, mittleres und hohes Ammonium/Nitrat Verhältnis aufwiesen (Kapitel 4 & 5). Gemessen wurde die Mykorrhiza-Kolonisation, die Trockenmasse und die N, P, K, S, NO_3^-, Mg und Zn Konzentrationen im Pflanzenspross.

Auf den Torf basierten Substraten ergab die Inokulation mit AM Pilzen eine Kolonisationsrate von bis zu 70% der Wurzellänge von Porree (Kapitel 1), 36% von Pelargonie und 2% von Poinsettien (Kapitel 3). Die AM Kolonisation erhöhte die Spross Zn und K Konzentrationen in Porree, die Spross P und K Konzentrationen in Pelargonie, aber die AM Kolonisation beeinflusste bei keiner der drei Spezies signifikant die Sprosstrokkenmasse oder N Konzentration im Spross. Ebenso unterstützte die AM Kolonisation (bis zu 65%) der Salatpflanzen (Kapitel 2) die Trockenmassebildung weder auf Substraten mit substrateigenem P, Rohphosphate (niedrige P Verfügbarkeit), oder gut löslichem P (gute P Verfügbarkeit) im Vergleich zu nicht mykorrhizierten Pflanzen. Während AM Kolonisation die Spross N, Mg und Zn Konzentrationen in Salatpflanzen erhöhte, wurde die Spross P Konzentration nicht erhöht (Kapitel 2). Kompostgaben zu

den Torf basierten Substraten (Kapitel 1 & 3) hingegen erhöhte das Pflanzenwachstum, die Spross P Konzentration und in Porree auch die Spross K Konzentration. Allerdings deckte die Zugabe von Kompost nicht immer den Nährstoffbedarf der Pflanzen. Eventuell wird P auf Torf basierten Substraten fixiert und ist dadurch weniger verfügbar für den AM Pilz als auf Böden. Dahingegen scheint K vorzugsweise auf Torf basierten Substraten mit Kompostgaben verfügbar zu sein. Auf Perlit war die AM Kolonisationsrate von *Allium fistulosum* (Kapitel 4) niedrig, aber die AM Kolonisation erhöhte die Trokkenmasse der Pflanzen drastisch bei hohen und mittleren Ammonium/Nitrat Verhältnissen (niedriger pH). Die AM Kolonisation hatte keinen Einfluß auf das Wachstum von *A. tuberosum* (Kapitel 5). Hier wurde die höchste Kolonisationsrate (43%) bei niedrigem Ammonium/Nitrat Verhältnis beobachtet, die mit steigendem Ammonium/Nitrat Verhältnis abnahm. Allgemein erhöhte die AM Kolonisation die Spross N, S und P Konzentrationen von *A. fistulosum* und die Spross N, P, K, Mg und NO_3^- Konzentrationen von *A. tuberosum* nicht signifikant. Nur die Spross NO_3^- Konzentrationen von *A. fistulosum* und die Spross S Konzentrationen von *A. tuberosum* wurden durch AM Kolonisation erhöht. Stickstoffgaben in Form eines hohen Ammonium/Nitrat Verhältnisses erhöhte die Spross N und P Konzentrationen von *A. fistulosum*. Die Trockenmasse bei *A. fistulosum* erhöhte sich bei einem niedrigen Ammonium/Nitrat Verhältnis im Vergleich zu den anderen beiden Ammonium/Nitrat Verhältnissen. Im Gegensatz dazu erreichte *A. tuberosum* gleiche Trockenmassen bei niedrigen und mittleren Ammonium/Nitrat Verhältnissen. Insgesamt war der Einfluss der AM Kolonisation auf die Nährstoffaufnahme aufgrund der hohen Nährstoffzufuhr variabel. Gegen Ammonium Toxizität konnte ein Schutz durch den AM Pilz nachgewisen werden, aber diese Ergebnisse waren nicht immer wiederholbar.

Der Effekt vom AM Pilz auf die Entwicklung von Knospen und Blüten wurde bei inokulierten Pelargonien und Poinsettien untersucht. Die Pflanzen wurden mit drei kommerziellen AM Inokula in Kombination mit 20% und 40% Kompostgaben auf Torf basierten Substraten untersucht (Kapitel 3). Die AM Kolonisation erhöhte die Anzahl der Knospen und Blüten bei beiden Pflanzenspezies.

Behandlungseffekte von AM Kolonisation auf die sekundären Pflanzestoffe von *A. fistulosum* und *A. tuberosum* wurden in Kombination mit drei verschiedenen Verhältnissen von Ammonium zu Nitrat in der Nährlösung auf Perlit untersucht. Die untersuchten Inhaltsstoffe waren Einzelzucker (Glukose, Fruktose, und Saccharose), lösliche Feststoffe und organische Schwefelverbindungen (gemessen als Pyruvat). In *A. fistulosum* (Kapitel 4) hatten das Ammonium/Nitrat Verhältnis und die AM Kolonisation, sowie in *A. tuberosum* (Kapitel 5) die AM Kolonisation kaum einen Einfluss auf die

Konzentrationen der Einzelzucker, der löslichen Feststoffe, oder des Pyruvats. Allerdings hatten *A. tuberosum* Pflanzen bei einem mittleren Verhältnis von Ammonium zu Nitrat erhöhte Pyruvat Konzentrationen im Vergleich zu Pflanzen, die auf einem niedrigen Ammonium/Nitrat Verhältnis wuchsen. Im Bezug auf Sprosswachstum und Inhaltsstoffe zeigten die nicht mykorrhizierten *A. fistulosum* Pflanzen erhöhte Trockenmassen und Gesamtgehalte bei einem niedrigen Ammonium/Nitrat Verhältnis im Vergleich zu den anderen Ammonium/Nitrat Verhältnissen. Jedoch produzierten *A. fistulosum* Pflanzen gleich hohe Gesamtgehalte von organischen Schwefelverbindungen bei niedrigen und mittleren Ammonium/Nitrat Verhältnissen, wenn sie von AM Kolonisation gegen den einschränkenden Effekt einer hohen Ammonium Düngung geschützt waren. Im Gegensatz dazu war das mittlere Ammonium/Nitrate Verhältnis für *A. tuberosum* die bevorzugte Mischung für die Produktion eines hohen Gesamtgehalts an gesundheitsfördernden organischen Schwefelverbindungen unabhängig von der AM Kolonisation.

Eine spontane Entwicklung von AM Kolonisation in den nicht mykorrhizierten Behandlungen aller verwendeter Substrate konnte nicht beobachtet werden, obwohl die Torf basierten Substrate und der Kompost vor Gebrauch nicht sterilisiert wurden. Nach einer Inokulation war jedoch eine hohe Kolonisationsrate erreichbar. Diese Beobachtung hat einen hohen praktischen Wert für den Gartenbauer. Für den Anbau von mykorrhizierten Pflanzen auf diesen Substraten ist eine AM Inokulation unumgänglich. Dies sollte mit einem niedrigen Nährstoffangebot gekoppelt sein.

Eine AM Kolonisation konnte die Nährstoffversorgung der Pflanze verbessern und die Blütenbildung erhöhen. Jedoch profitierten die Pflanzen unter den beschriebenen experimentellen Bedingungen nicht durchgängig in ihrem Wachstum und ihren Inhaltstoffen von dem AM Pilz. Die Zugabe von Kompost ermöglichte die Verbesserung der Substratqualität für die Nährstoffversorgung und das Pflanzenwachstum unter biologischen Gartenbaubedingungen. Der Ertrag von gesundheitsförderlichen organischen Schwefelverbindungen und die damit verbundene Pflanzenqualität konnten, in Abhängigkeit von der jeweiligen *Allium* Spezies, durch eine Variation des Ammonium/Nitrat Verhältnisses oder einen AM Effekt auf das Wachstum, gesteigert werden.

Reference List

Aboul-Nasr, A. (1996): Effects of vesicular-arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*, Mycorrhiza (volume 6), pp. 61-64.

Ahlers, W. (2001): Brix-Values, <http://www.kennzeichnungsrecht.de/pseudoanzeige3.html?glossar.htm?a2_brix.html> (18.05.06).

Akiyama, K.; Matsuzaki, K.-I. and Hayashi, H. (2005): Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi, Nature. (volume 435), pp. 824-827.

Allen, M. F.; Moore, T. S. and Christensen, M. (1982): phytohormone changes in Bouteloua gracilis infected by vesicular-arbuscular mycorrhizae. II. Altered levels of gibberellin-like substances and abscisic acid in the host plant, National Research Council of Canada, pp. 468-471.

Alloush, G. A. and Clark, R. B. (2001): Maize response to phosphate rock and arbuscular mycorrhizal fungi in acidic soil, Communication in Soil Science and Plant Analysis (volume 32), issue 1-2, pp. 231-254.

Andrade, S. A. L.; Abreu, C. A.; de Abreu, M. F. and Silveira, A. P. D. (2004): Influence of lead additions on arbuscular mycorrhiza and *Rhizobium* symbioses under soybean plants, Applied Soil Ecology (volume 26), pp. 123-131.

Andrews, M. (1986): Nitrate and reduced-N concentrations in the xylem sap of Stellaria media, Xanthium strumarinum and six legume species, Plant, Cell and Environment (volume 9), pp. 605-608.

Anonymus (1998): Verordnung über die Verwertung von unbehandelten und behandelten Bioabfällen auf landwirtschaftlich, forschwirtschaftlich und gärtnerisch genutzten Böden, Bioabfallverordnung-BioAbfV.

Asami, D. K.; Hong, Y. J.; Barret, D. M. and Mitchell, A. E. (2003): Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices, Journal of Agricultural Food Chemistry (volume 51), pp. 1237-1241.

Asghari, H. R.; Chittleborough, D. J.; Smith, F. A. and Smith, S. E. (2005): Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores, Plant and Soil (volume 275), pp. 181-193.

Aslam, M.; Travis, R. L. and Huffacker, R. (1992): Comparative kinetics and reciprocal inhibition of nitrate and nitrite uptake in roots of uninduced and induced barley (*Hordeum vulgare* L.) seedlings, Plant Physiology (volume 99), pp. 1124-1133.

Azcón, R.; Ambrosano, E. and Charest, C. (2003): Nutrient aquisition in mycorrhizal lettuce plants under different phosphorus and nitrogen concentration, Plant Science (volume 165), pp. 1137-1145.

Azcón-Aguilar, C. and Barea, J. M. (1997): Applying mycorrhiza biotechnology to horticulture: significance and potentials, Scientia Horticulturae (volume 68), pp. 1-24.

Baake, K.-H (2006): Der Biogärtner, <http://bio-gaertner.de/Articles/index.html> (18.06.06).

Backhaus, G. F. (1983): Einfluss der vesikulären-arbusculären Mykorrhiza auf die generative Entwicklung von Heliotropium und Fuchsia, Gartenbauwissenschaften (volume 48), issue 5, pp. 197-201.

Bago, B. and Azcón-Aguilar, C. (1997): Changes in the rhizospheric pH induced by arbuscular mycorrhiza formation in onion (*Allium cepa* L.), Zeitung für Pflanzenernährung und Bodenkunde (volume 160), pp. 333-339.

Balestrini, R. and Bonfante, P. (2005): The interface compartment in arbuscular mycorrhizae: A special type of plant cell wall?, Plant Biosystems (volume 139), issue 1, pp. 8-15.

Balestrini, R.; Cosgrove, D. J. and Bonfante, P. (2005): Differential location of á-expansin proteins during the accommodation of root cells to an arbuscular mycorrhizal fungus, Planta (volume 220), pp. 889-899.

Barea, J. M.; Azcón, R. and Haman, D. S. (1975): Possible synergetic interactions between Endogone and phosphate solubilizing bacteria in low phosphate soils, Sanders, F. E.; Mosse, B. and Tinker, P. B., pp. 409-417, Academic Press, London, UK, Endomycorrhizas.

Barea, J. M.; Pozo, M. J. and Azcón-Aguilar, C. (2005): Microbial co-operation in the rhizosphere, Journal of Experimental Botany. (volume 56), issue 417, pp. 1761-1778.

Barea, J. M.; Tot, M.; Orozco, M. O.; Campos, E. and Azcón, R. (2002): The application of isotopic (^{23}P and ^{15}N)dilution techniques to evaluate the interactive effect of phosphate-solubilizing rhizobacteria, mycorrhizal fungi and *Rhizobium* to improve the agronomic efficiency of rock phosphate for legume crops, Nutrient Cycling in Agroecosystems (volume 63), pp. 35-42.

Barker, S. J. and Tagu, D. (2000): The role of auxins and cytokinins in mycorrhizal symbioses, Journal of Plant Growth Regulation (volume 19), pp. 144-154.

Baylis, G. T. S. (1972): Fungi, phosphorus, and the evolution of root systems, Search (volume 3), issue 7, pp. 257-258.

Bénkova, E.; Michniewicz, M.; Sauer, M.; Teichmann, T.; Seifertová, D.; Jürgens, G. and Frimi, J. (2003): Local, efflux-dependent auxin gradients as a common module for plant organ formation, Cell (volume 115), pp. 591-602.

Berger, H.; Pachlinger, R.; Morozov, I.; Goller, S.; Narendja, F; .; Caddick, M. and Strauss, J. (2006): The GATA factor AreA reguloates localization and in vivo binding site occupancy of the nitrate activator NirA, Molecular Microbiology (volume 59), issue 2, pp. 433-446.

Bergès, R.; Siess, M. H.; Arnault, I.; Auger, J.; Kahane, R.; Pinnert, M. F.; Vernevaut, M. F. and Le Bon, A. M. (2004): Comparison of the chemopreventive efficacies of garlic powders with different alliin contents against aflatoxin B1 carcinogenicity in rats, Carcinogenesis (volume 25), issue 10, pp. 1953-1959.

Bergmann, W. (1993): Ernährungsstörungen bei Kulturpflanzen, 3rd. edition, Gustav Fischer Verlag, Stuttgart, Germany.

Besmer, Y. L. and Koide, R. T. (1999): Effect of mycorrhizal colonization and phosphorus on ethylene production by snapdragon (*Antirrhinum majus* L.) flowers, Mycorrhiza (volume 9), pp. 161-166.

Bialczyk, J.; Lechowski, Z.; Dziga, D. and Molenda, K. (2005): Carbohydrate and free amino acid contents in tomato plants grown in media with bicarbonate and nitrate or ammonium, Acta Physiologiae Plantarum (volume 27), issue 4A, pp. 523-529.

Bioland (2005): Richtlinien, <http://www.bioland.de/bioland/richtlinien.html> (18.05.06).

Bloem, E.; Haneklaus, S. and Schnug, E. (2005): Significance of sulfur compounds in the protection of plants against pests and diseases, Journal of Plant Nutrition (volume 28), pp. 763-784.

Bloom, A. J.; Sukrapanna, S. S. and Warner, R. L. (1992): Root respiration associated with ammonium and nitrate absorption and assimilation by barley, Plant Physiology (volume 99), pp. 1294-1301.

Boddington, C. L. and Dodd, J. C. (2000): The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. II. Studies in experimental microcosms., Plant and Soil (volume 218), issue 1-2, pp. 145-157.

Bonfante, P. (2003): Plants, mycorrhizal fungi and endobacteria: a dialog among cells and genomes, The Biolobical Bulletin (volume 204), pp. 215-220.

Breuninger, M.; Trujillo, C. G.; Serrano, E.; Fischer, R. and Requena, N. (2004): Different nitrogen sources modulate activity but not expression of glutamine synthetase in arbuscular mycorrhizal fungi, Fungal Genetics and Biology (volume 41), pp. 542-552.

Brewster, J. L. (1994): Onions and other Vegetable Alliums, Atherton J and Rees A, CAB International, Oxon, UK.

Britto, D. T. and Kronzucker, H. J. (2002): NH_4^+ toxicity in higher plants: a critical review, Journal of Plant Physiology (volume 159), issue 6, pp. 567-587.

Britto, D. T. and Kronzucker, H. J. (2005): Nitrogen acquisition, PEP carboxylase, and cellular pH homeostasis: new views on old paradigms, Plant, Cell and Environment (volume 28), issue 11, pp. 1396-1409.

Britto, D. T.; Ruth, T. J.; Lapi, S. and Kronzucker, H. J. (2004): Cellular and whole-plant chloride dynamics in barley: insights into chloride-nitrogen interactions and salinity responses, Planta (volume 218), pp. 615-622.

Britto, D. T.; Siddiqi, M. Y.; Glass, A. D. M. and Kronzucker, H. J. (2002): Subcellular NH_4+ flux analysis in leaf segments of wheat (*Triticum aestivum*), New Phytologist (volume 155), pp. 373-380.

Bruce, A.; Smith, S. E. and Tester, M. (1994): The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units, New Phytologist (volume 127), pp. 507-514.

Brundrett, M. C. (2002): Coevolution of roots and mycorrhizas of land plants, New Phytologist (volume 154), issue 2, pp. 275-304.

Brunold, C. and Suter, M. (1984): Regulation of sulfate assimilation by nitrogen nutrition in the duckweed Lemna minor L., Plant Physiol. (volume 76), pp. 579-583.

Bruns, C. (1998): Suppressive Effekte von Komposten aus der getrennten Sammlung organischer Abfälle und von Rindenkompost gegenüber bodenbürtigen Schaderregern, Univ.Kassel, Pahl Rugenstein Hochschulschriften 293, Bonn.

Bruns, C. and Schüler, C. (28-8-2000): Suppressive effects of yard waste compost amended growing media on soilborne plant pathogens in organic horticulture Basel, Switzerland.

Bücking, H. and Shachar-Hill, Y. (2005): Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability., New Phytologist (volume 165), issue 899, pp. 912.

Bugbee, B. G. and Salisbury, F. B. (1985): An evaluation of MES (2(N-morpholino)ethansulfonic acid) and amberlite IRC-50 as pH buffers for nutrient solution studies, Journal of Plant Nutrition (volume 8), issue 7, pp. 567-583.

Cantwell, M.; Nie, X.; Zong, R. J. and Yamaguchi, M. (1996): Asian vegetables: Selected fruit and leafy types, Janick J, Progress in new crops , pp. 488-495, ASHS Press, Arlington, VA.

Carpenter-Boggs, L.; Kennedy, A. C. and Reganold, J. P. (2000): Organic and biodynamic management: effect on soil biology, Soil Sci.Soc.Am J. (volume 64), pp. 1651-1659.

Chen, B. D.; Roos, P.; Borggaard, O. K.; Zhu, Y. G. and Jakobsen, I. (2005): Mycorrhiza and root hairs in barley enhance acquisition of phosphorus and uranium from phosphate rock but mycorrhiza decreases root to shoot uranium transfer, New Phytologist (volume 165), pp. 591-598.

Clark, R. B. and Zeto, S. K. (2000): Mineral acquisition by arbuscular mycorrhizal plants, Journal of Plant Nutrition (volume 23), issue 7, pp. 867-902.

Clarkson, D. T.; Saker, L. R. and Purves, J. V. (1989): Depression of nitrate and ammonium transport in barley plants with diminished sulphate status. Evidence of Co-regulation of nitrogen and sulphate intake, Journal of Experimental Botany. (volume 40), issue 218, pp. 953-963.

Claussen, W. and Lenz, F. (1995): Effect of ammoinum and nitrate on net photosynthesis, flower formation, growth and yield of eggplants (*Solanum melongena* L.), Plant and Soil (volume 171), pp. 267-274.

Coolong, T. W. and Randle, W. M. (2003): Sulfur and nitrogen availability interact to affect the flavor biosynthetic pathway in onion, Journal of the American Society for Horticultural Science. (volume 128), issue 5, pp. 776-783.

Cooper, R. M. and Williams, J. S. (2004): Elemental sulphur as a induced antifungal substance in plant defence, Journal of Experimental Botany. (volume 55), issue 404, pp. 1947-1953.

Craine, J. M.; Fargoine, J. and Sugita, S. (2005): Supply pre-emption, not concentration reduction, is the mechanism of competition for nutrients, New Phytologist (volume 166), pp. 933-940. URL: Joseph M. Craine[1], Joseph Fargione[2] and Shinya Sugita[3]

Curatti, L.; Giarrocco, L. and Salerno, G. L. (2006): Sucrose synthase and RuBisCo expression is similarly regulated by the nitrogen source in the nitrogen-fixing cyanobacterium *Anabaena* sp., Planta (volume 223), pp. 891-900.

Danneberg, G.; Latus, C.; Zimmer, W.; Hundeshagen, B.; Schneider-Poetsch, H. J. and Bothe, H. (1992): Influence of vesicular-arbuscular mycorrhiza on phytohormone balances in maize (*Zea mays* L.), Journal of Plant Physiology (volume 141), pp. 33-39.

Date, S.; Terabayashi, S.; Kobayashi, Y. and Fujime, Y. (2005): Effects of chloramines concentration in nutrient solution and exposure time on plant growth in hydroponically cultured lettuce, Scientia Horticulturae (volume 103), pp. 257-265.

Demchenko, K.; Winzer, T.; Stougaard, J.; Parniske, M. and Pawlowski, K. (2004): Distinct roles of Lotus japonicus SYMRK and SYM15 in root colonization and arbuscule formation, New Phytologist (volume 163), pp. 381-392.

Demeter (2004): Richtlinien für die Anerkennung der Demeter-Qualität, <http://www.demeter.de/> (18.05.06).

Dickson, S.; Smith, S. E. and Smith, F. A. (1999): Characterisation of two arbuscular mycorrhizal fungi in symbiosis with *Allium porrum*: colonization, plant growth and phosphate uptake, New Phytologist (volume 144), issue 1, pp. 163-172.

Douds, D. D.; Nagahashi, G.; Pfeffer, P. E.; Kayser, W. M. and Reider, C. (2005): On-farm production and utilization of arbuscular mycorrhizal fungus inoculum, Can.J.Plant Sci (volume 85), pp. 15-21.

Douds, D. D. Jr. and Reider, C. (2003): Inoculation with Mycorrhizal fungi increases the yield of green peppers in a high P soil, Biological Argiculture and Horticulture (volume 21), pp. 91-102.

Drew, E. A.; Murray, R. S. and Smith, S. E. (2003): Beyond the rhizosphere: growth and function of arbuscular mycorrhizal external hyphae in sands of varying pore sizes, Plant and Soil (volume 251), issue 1, pp. 105-114.

Dumas-Gaudot (2000): Modulation of host defence systems, Kapulnik, Y and Douds, DD Jr, Arbuscular Mycorrhizas: Physiology and Function , pp. 173-200, Kluwer Academic Publisher.

Duponnois, R.; Colombet, A.; Hien, V. and Thioulouse, J. (2005): The mycorrhizal fungus *Glomus interaradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*, Soil Biology & Biochemistry (volume 37), issue 1460, pp. 1468.

Durenkamp, M. and De Kok, L. J. (2002): The impact of atmospheric H2S on growth and sulfur metabolism of Allium cepa L., Phyton-Annales rei Botanicae. (volume 42), issue 3, pp. 55-63.

Durenkamp, M. and De Kok, L. J. (2004): Impact of pedospheric and atmospheric sulphur nutrition on sulphur metabolism of Allium cepa L., a species with a potential sink capacity for secondary sulphur compounds, Journal of Experimental Botany. (volume 55), issue 404, pp. 1821-1830.

Ecke, P.; Matkin, O. A. and Hartley, D. E. (1990): The poinsettia Manual, 3. edition, Paul Ecke Poinsettia, Encinitas, California, USA.

El Dessougi, H.; zu Dreele, A. and Claassen, N. (2003): Growth and phosphorus uptake of maize cultivated alone, in mixed culture with other crops or after incorporation of their residues, Journal of Plant Nutrition and Soil Science (volume 166), pp. 254-261.

Elsner, W.; Loeser, H. and Biermann, W. (1995): Gärtnerhandbuch Pelargonie, Ernst Thalacker Verlag, Braunschweig.

Encyclopedia (2003): Lettuce: The Columbia Electronic Encyclopedia, <http://www.answers.com/library/Encyclopedia> (18.05.06), issue 6.

Espoma (2006): Rock Phosphate, <http://www.espoma.com/> (18.06.06).

European Union (2004): Council Regulation No 2092/91/EEC of 24 June 1991 on organic production of agricultural products and indications referring thereto on agricultural products and foodstuffs. 24 Feb. 05., <http//europa.eu.int/scadplus/leg/en/lvb/l21118.htm> (18.05.06).

FAO (1972): Food composition table for use in east asia, <http://www.fao.org/DOCREP/003/X6878E/X6878E00.htm> (18.05.06).

Feng, G.; Song, Y. C.; Li, X. L. and Christie, P. (2003): Contribution of arbuscular mycorrhizal fungi to utilization of organic ources of phosphorus by red clover in a calcerous soil, Applied Soil Ecology (volume 22), pp. 139-148.

Fenwick, G. R. and Hanley, A. B. (1985): The genus *Allium*-part 3, CRC Crit.Rev.Food Sci.Nutr. (volume 23), pp. 1-73.

Ferrario-Méry, S.; Bouvet, M.; Leleu, O.; Savino, G.; Hodges, M. and Meyer, C. (2005): Physiological characterisation of Arabidopsis mutants affected in the expression of the putative regulatory protein PII, Planta (volume 223), pp. 28-39.

Fitze, D.; Wiepning, A.; Kaldorf, M. and Ludwig-Müller, J. (2005): Auxins in the development of an arbuscular mycorrhizal symbiosis in maize, Journal of Plant Physiology (volume 162), pp. 1210-1219.

Frachisse, J.-M.; Thomine, S.; Colcombet, J.; Guern, J. and Barbier-Brygoo, H. (1999): Sulfate Is Both a Substrate and an Activator of the Voltage-Dependent Anion Channel of *Arabidopsis* Hypocotyl Cells[1], Plant Physiology (volume 121), pp. 253-262.

Frossard, E.; Skrabal, P.; Sinaj, S.; Bangerter, F. and Traore, O. (2002): Forms and exchangeability of inorganic phosphate in composted solid organic wastes, Nutrient Cycling in Agroecosystems (volume 62), pp. 103-113.

Fusconi, A.; Lingua, G.; Trotta, A. and Berta, G. (2005): Effects of arbuscular mycorrhizal colonization and phosphorus application on nuclear ploidy in *Allium porrum* plants, Mycorrhiza (volume 15), pp. 313-321.

Gahoonia, T. S. and Nielsen, N. E. (1998): Direct evidence on participation of root hairs in phosphorus (^{32}P) uptake from soil, Plant and Soil (volume 198), pp. 147-152.

Gaur, A. and Adholeya, A. (2005): Diverse Response of five ornamental plant species to mixed indigenous and single isolate arbuscular-mycorrhizal inocula in marginal soil amended with organic matter, Journal of Plant Nutrition (volume 28), pp. 707-723.

Genre, A.; Chabaud, M.; Timmers, T.; Bonfante, P. and Barker, D. G. (2005): Arbuscular mycorrhizal fungi elicit a novel intracellular apparatus in *Medicago truncatula* root epidermal cells before infection, The Plant Cell (volume 17), pp. 3489-3499.

George, E. (2000): Nutrient uptake. Contribution of arbuscular mycorrhizal fungi to plant mineral nutrition, Kapulnik, Y and Douds, DD Jr, Arbuscular Mycorrhizas: Physiology and Function , pp. 307-343, Kluwer Academic Publisher.

George, E. and Eghbal, R. (2003): Ökologischer Gemüsebau: Handbuch für Beratung und Praxis, Bioland Verlag, Mainz.

George, E.; Häussler, K. U.; Vetterlein, D.; Gorgus, E. and Marschner, H. (1992): Water and nutrient translocation by hyphae of *Glomus mosseae*, Canadian Journal of Botany (volume 70), pp. 2130-2137.

Gilroy, S. and Jones, D. L. (2000): Through form to function: root hair development and nutritient uptake, Trends in Plant Science (volume 5), issue 2, pp. 56-60.

Giovannetti, M. and Mosse, B. (1980): An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots, New Phytologist (volume 84), pp. 489-500.

Glass, A. D. M. and Siddiqi, M. Y. (1995): Nitrogen absorption by plant roots. *In* Nitrogen nutrition in higher plants, Srivastava H and Singh R, pp. 21-56, Associated Publishing Company, New Dehli, India.

Goldman, I. L.; Kopelberg, M.; Debaene, J. E. P. and Schwartz, B. S. (1996): Antiplatelet activity in onion (Allium cepa) is sulfur dependent, Thrombosis and Haemostasis (volume 76), issue 3, pp. 450-452.

Gosling, P.; Hodge, A.; Goodlass, G. and Bending, G. D. (2006): Arbucular mycorrhizal fungi and organic farming, Agriculture, Ecosystems and Environment (volume 113), pp. 17-35.

Goswani, M. G. and Jamaluddin (2001): A note on vesicular-arbuscular mycorrhiza development in *Alizia procera* in root trainers, Mycorrhiza News (volume 13), issue 1, pp. 21-22.

Govindarajulu, M.; Pfeffer, P. E.; Jin, H.; Abubaker, J.; Douds, D. D.; Allen, J. W.; Bücking, H.; Lammers, P. J. and Shachar-Hill, Y. (2005): Nitrogen transfer in the arbuscular mycorrhizal symbiosis, Nature. (volume 435), pp. 819-823.

Granstedt, R. C. and Huffaker, R. C. (1982): Identification of the leaf vacuole as a major nitrate storage pool, Plant Physiology (volume 70), pp. 410-413.

Green, N. E.; Graham, S. O. and Schenck, N. C. (1976): The influence of pH on the germination of versicular-arbuscular mycorrhizal spores, Mycologia (volume 68), pp. 929-934.

Grey, M. and Henry, C. (1999): Nutrient retention and release characteristics from municipal soild waste compost, Compost Science and Utilization (volume 7), issue 1, pp. 42-50.

Griffiths, G.; Trueman, L.; Crowther, T.; Thomas, B. and Smith, B. (2002): Onions-a global benefit to health, Phytotherapy Research (volume 16), pp. 603-615.

Grimoldi, A. A.; Kavanová, M.; Lattanzi, F. A. and Schnyder, H. (2005): Phosphorus nutrition-mediated effects of arbuscular mycorrhiza on leaf morphology and carbon allocation in perennial ryegrass, New Phytologist (volume 168), pp. 435-444.

Gryndler, M.; Hrselová, H.; Sudová, R.; Gryndlerová, H.; Rezácová, V. and Merhautová, V. (2005a): Hyphal growth and mycorrhiza formation by the arbuscular mycorrhizal fungus *Glomus claroideum* BEG 23 is stimlated by humic substances, Mycorrhiza (volume 15), pp. 483-488.

Gryndler, M.; Larsen, J.; Hrselová, H.; Rezácová, V.; Gryndlerová, H. and Kubát, J. (2005b): Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment, Mycorrhiza (volume 16), issue 3, pp. 159-166.

Hams, S. and Becker, G.: State of the Art of Source Separation, Collection and Treatment of Organic Waste in Germany <http://www.environmental-center.com/articles/article718/article718.htm> (17.06.06).

Hause, B. and Fester, T. (2005): Molecular and cell biology of arbuscular mycorrhizal symbiosis, Planta (volume 221), pp. 184-196.

Hause, B.; Maier, W.; Miersch, O.; Kramell, R. and Strack, D. (2002): Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots, Plant Physiology (volume 130), pp. 1213-1220.

Hawkesford, M. J. (2003): Transporter gene families in plants: the sulphate transporter gene family - redundancy or specialization, Physiologia Plantarium (volume 117), pp. 155-163.

Hawkesford, M. J. and Wray, J. L. (2000): Molecular genetics of sulphate assimilation, Callow JA, Advances in botanical research incorporating advances in plant pathology , pp. 159-223, Academic Press Ltd, London, UK.

Hawkins, H. J. and George, E. (2001): Reduced N-15-nitrogen transport through arbuscular mycorrhizal hyphae to Triticum aestivum L. supplied with ammonium vs. nitrate nutrition, Annals of Botany. (volume 87), issue 3, pp. 303-311.

Hawkins, H. J. and George, E. (1999): Effect of plant nitrogen status on the contribution of arbuscular mycorrhizal hyphae to plant nitrogen uptake, Physiologia Plantarium (volume 105), pp. 694-700.

Hawkins, H. J.; Johansen, A. and George, E. (2000): Uptake and transport of organic and inorganic nitrogen by arbuscular mycorrhizal fungi, Plant and Soil (volume 226), pp. 275-285.

Haymann, D. S. and Mosse, B. (1972): Plant growth responses to vesicular-arbuscular mycorrhiza. III. Increased uptake of labile P from soil, New Phytol. (volume 71), pp. 41-47.

He, Y.; Michaels, S. D. and Amasino, R. M. (2003): Regulation of flowering time by histone acetylation in *Arabidopsis*, Science (volume 302), pp. 1751-1754.

Hell, R. (1997): Molecular physiology of plant sulfur metabolism, Planta (volume 202), pp. 138-148.

Herrmann, G. and Plakolm, G. (1991): Ökologischer Landbau Grundwissen für die Praxis, Österreichischer Agrarverlag, Wien, Austria.

Heß, D. (1999): Pflanzenphysiologie. Molekulare und biochemische Grundlagen von Stoffwechsel und Entwicklung der Pflanze, 10. edition, Ulmer, Stuttgart.

Hesse, H. and Hoefgen, R. (2003): Molecular aspects of methionine biosynthesis, Trends in Plant Science (volume 8), issue 6, pp. 259-262.

Hesse, H.; Nikiforova, V. J.; Gakiere, B. and Hoefgen, R. (2004): Molecular analysis and control of cysteine biosynthesis: intergration of nitrogen and sulphur metabolism, Journal of Experimental Botany. (volume 55), issue 401, pp. 1283-1292.

Hoagland, D. R. and Arnon, D. I. (1938): The water-culture methode for growing plants without soil (volume Circular 347), University of California, College of Agriculture, Berkly.

Ibanez, M. D.; Fernandez-Nieto, M.; Martinze, J.; Cardona, G. A.; Guisantes, J.; Quirce, S. and Sastre, J. (2004): Asthma induced by latex from 'Christmas flower' (Euphorbia pulcherrima), Allergy (volume 59), issue 10, pp. 1127-1128.

Imai, S.; Tsuge, N.; Tomotake, M.; Nagatome, Y.; Sawada, H.; Nagata, T. and Kumugai, H. (2002): An onion enzyme that makes the eyes water - A flavoursome, user-friendly bulb would give no cause for tears when chopped up, Nature. (volume 419), issue 6908, pp. 685-685.

Jack, T. (2004): Molecular and genetic mechanisms of floral control, The Plant Cell (volume 16), pp. S1-S17.

Jakobsen, I.; Chen, B.; Munkvold, L.; Lundsgaard, T. and Zhu, Y.-G. (2005): Contrasting phosphate acquisition of mycorrhizal fungi with that of root hairs using the root hairless barley mutant, Plant, Cell and Environment (volume 28), pp. 928-938.

Jakobsen, I.; Joner, E. J. and Larsen, J. (1994): Hyphal phosphorus transport, a keystone to mycorrhizal enhancement of plant growth, Gianinazzi, S and Schüepp, H,

Impact of Arbuscular Mycorrhizas on sustainable agriculture and natural ecosystems , pp. 133-146, Birkhäuser Verlag, Basel.

Johnston, A. E. and Steén, I. (2000): Understanding Phosphorus and its Use in Agriculture, <http://www.efma.org/index.asp> (18.05.06).

Joner, E. J. and Johansen, A. (2000): Phosphatase activity of external hyphae of two arbuscular mycorrhizal fungi, Mycological Research (volume 104), issue 1, pp. 81-86.

Jones, M. G.; Hughes, J.; Tregova, A.; Milne, J.; Tomsett, A. B. and Collin, H. A. (2004): Biosythesis of the flavour precursors of onion and garlic, Journal of Experimental Botany. (volume 55), issue 404, pp. 1903-1918.

Joosten, H. and Clarke, D. (2002): Wise use of mires and peatlands - background and principles including a framework for decision-making, International Mire Conservation Group and International Peat Society, Saarijärvi, Finnland.

Kafkafi, U. (1990): Root temperature, concentration and the ration NO_3^-/NH_4^+ effect on plant development, Journal of Plant Nutrition (volume 13), issue 10, pp. 1291-1306.

Kasiamdari, R. S.; Smith, S. E.; Smith, F. A. and Scott, E. S. (2002): Influence of the mycorrhizal fungus, *Glomus coronatum*, and soil phosphorus on infection and disease caused by binucleate *Rhizoctonia* and *Rhizoctonia solani* on mung bean (*Vigna radiata)*, Plant and Soil (volume 238), pp. 235-244.

Keiss, H. P.; Dirsch, V. M.; Hartung, T.; Haffner, T.; Trueman, L.; Auger, J.; Kahane, R. and Vollmar, A. (2003): Garlic (*Allium sativum* L.) modulates cytokine expression in lipopolysaccharide-activated human blood thereby inhibiting NF-$_k$B activity, American Society for Nutritional Sciences. (volume 133), pp. 2171-2175.

Ketter, C. A. T. and Randle, W. M.: Pungency assessment in onion (19) Proceedings of the 19th Workshop/Conference of the Association for Biology Laboratory Education (ABLE).URL: http://www.zoo.utoronto.ca/able/volumes/vol-19/11-randle/11-randle.htm

Kitazawa (2006): Bunching onion, <http://www.kitazawaseed.com/seeds_bunching_onion.html> (18.05.06).

Kodera, Y.; Matsuura, H.; Sumiyoshi, H. and Sumi, S. (2003): Garlic chemistry: Chemical and biological properties of sulfur-containing compounds derived from garlic, Food facors in health promotion and disease prevention , pp. 346-357, American Chemical Society, Washington.

Köhler, B.; Wegner, L. H.; Osipov, V. and Raschke, K. (2002): Loading of nitrate into the xylem: apoplastic nitrate controls the voltage dependence of X-QUAC, the main anion conductance in xylem-parenchyma cells of barley roots, Plant Journal (volume 30), issue 2, pp. 133-142.

Koide, R. T. (2000): Functional complementarity in the arbuscular mycorrhizal symbiosis, New Phytol. (volume 147), pp. 233-235.

Koide, R. T. and Kabir, Z. (2000): Extraradical hyphae of the mycorrhizal fungus *Glomus intraradices* can hydrolyse organic phosphate, New Phytologist (volume 148), pp. 511-517.

Koide, R. T. and Mosse, B. (2004): A history of research on arbuscular mycorrhiza, Mycorrhiza (volume 14), issue 3, pp. 145-163.

König, U. J. (2004): Development of criteria and methods for assessing the production and use of cultivation substrates for organic seedling production with particular consideration of the further development of regulation (EWG) 2092/91, <http://www.orgprints.org/5226> (18.05.06).

Kopriva, S.; Suter, M.; Von Ballmoos, P.; Hesse, H.; Krähenbühl, U.; Rennenberg, H. and Brunold, C. (2002): Interaction of sulfate assimilation with carbon and nitrogen in *Lemna minor*, Plant Physiology (volume 130), pp. 1406-1413.

Koprivova, A.; Suter, M.; Op den Camp, R.; Brunold, C. and Kopriva, S. (2000): Regulation of sulfate assimilation by nitrogen in Arabidopsis, Plant Physiology (volume 122), pp. 737-746.

Koske, R. E. and Gemma, J. N. (1989): A modified procedure for staining roots to detect VA mycorrhizas, Mycological Research (volume 92), issue 4, pp. 486-505.

Krizek, B. A. and Fletcher, J. C. (2005): Molecular mechanisms of flower development: an armchair guide, Nature Reviews Genetic. (volume 6), pp. 688-698.

Kronzucker, H. J.; Glass, A. D. M. and Siddiqi, M. Y. (1999a): Inhibition of Nitrate Uptake by Ammonium in Barley. Analysis of Component Fluxes, Plant Physiology (volume 120), issue 1, pp. 283-292.

Kronzucker, H. J.; Glass, A. D. M.; Siddiqi, M. Y. and Kirk, G. J. D. (1999b): Nitrate-ammonium synergism in rice: A subcellular analysis, Plant Physiology (volume 119), pp. 1041-1046.

Larkom, J. (1994): Vegetables on trial., New Farmer & Grower (volume 41), pp. 20-23.

Lawson, L. D. (1993): Bioactive organosulfur compounds of garlic and garlic products; Role in reducing blood lipids, Human medicinal agents from plants , American Chemical Society.

Lejay, L.; Gansler, X.; Cerezo, M.; Tillard, P.; Müller, C.; Krapp, A.; Von Wirén, N.; Daniel-Vedele, F. and Gojon, A. (2003): Regulation of root ion transporters by photosynthesis: Functional importance and relation with hexokinase, The Plant Cell (volume 15), pp. 2218-2232.

Lekberg, Y. and Koide, R. T. (2005): Is plant performance limited by abundance of arbuscular mycorrhizal fungi? A meta-analysis of studies published between 1988 and 2003, New Phytologist (volume 168), pp. 189-204.

Lerat, S.; Lapointe, L.; Piché, Y. and Vierheilig, H. (2003): Variable carbon-sink strength of different *Glomus mosseae* strains colonizing barley roots, Canadian Journal of Botany (volume 81), pp. 886-889.

Leung, H. M.; Ye, Z. H. and Wong, M. H. (2006): Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils, Environmental Pollution (volume 139), pp. 1-8.

Leustek, T.; Martin, M. N.; Bick, J. A. and Davies, J. P. (2000): Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies, Annual Review of Plant Physiology and Plant Molecualr Biology. (volume 51), pp. 141-165.

Leustek, T. and Saito, M. (1999): Sulfate transport and assimilation in plants, Plant Physiology (volume 120), pp. 637-643.

Li, X. L.; Marschner, H. and George, E. (1991): Acquisition of phosphorus and copper by VA-mycorrhizal hyphae and root-to-shoot transport in white clover, Plant and Soil (volume 136), pp. 49-57.

Linderman, R. G. and Davis, E. A. (2004): Evaluation of commercial inorganic and organic fertilizer effects on arbuscular mycorrhizae formed by *Glomus intraradices*, HortTechnology (volume 14), issue 2, pp. 196-202.

Linderman, R. G. and Davis, E. A. (2003a): Soil amendment with different peatmosses affects mycorrhizae of onion, HortTechnology (volume 13), issue 2, pp. 285-289.

Linderman, R. G. and Davis, E. A. (2001): Vesicular-arbuscular mycorrhiza and plant growth response to soil amendment with composted grape pomace or its water extract, HortTechnology (volume 11), issue 3, pp. 446-450.

Linderman, R. G. and Davis, E. A. (2003c): Arbusular mycorrhiza and growth responses of several ornamental plants grown in soilless peat-based medium amended with coconut dust (coir), HortTechnology (volume 13), issue 3, pp. 482-487.

Linderman, R. G. and Davis, E. A. (2003b): Arbusular mycorrhiza and growth responses of several ornamental plants grown in soilless peat-based medium amended with coconut dust (coir), HortTechnology (volume 13), issue 3, pp. 482-487.

Little, D.; Rao, H.; Oliva, S.; Daniel-Vedele, F.; Krapp, A. and Malamy, J. E. (2005): The putative high-affinity nitrate transporter NRT2.1 represses lateral root initiation in response to nutritional cues, PNAS (volume 102), issue 38, pp. 13693-13698.

Liu, A.; Wang, B. and Hamel, C. (2004): Arbuscular mycorrhiza colonization and development at suboptimal root zone temperature, Mycorrhiza (volume 14), pp. 93-101.

Lucassen, E. C. H. E. T.; Bobbink, R.; Smolders, A. J. P.; Van der Ven, P. J. M.; Lamers, L. P. M. and Roelofs, J. G. M. (2003): Interactive effects of low pH and high ammonium levels responsible for the decline of *Cirsium dissectum* (L.), Hill Plant Ecol (volume 165), pp. 45-52.

Ludwig-Müller, J.; Kaldorf, M.; Sutter, E. and Epstein, E. (1997): Indole-3-butyric acid (IBA) is enhanced in young maize (*Zea mays* L.) roots colonized with the arbuscular mycorrhizal fungus *Glomus intraradices*, Plant Science (volume 125), pp. 153-162.

Lüttge, U.; Kluge, M. and Bauer, G. (1999): Botanik, 3. edition, Wiley-VCH, Weinheim.

Ma, Z.; Bielenberg, D. G.; Brown, K. M. and Lynch, J. P. (2001): Regulation of root hair density by phosphorus availability in Arabidopsis thaliana, Plant, Cell and Environment (volume 24), pp. 459-467.

Mäder, P.; Fließbach, A.; Dubois, D.; Gunst, L.; Fried, P. and Niggli, U. (2002): Soil fertility and biodiversity in organic farming, Science (volume 296), pp. 1694-1697.

Maldonado-Mendoza, I.; Dewbre, G. R. and Harrison, M. J. (2001): A phosphate transporter gene from the extra-radical mycelium of an arbuscular mycorrhizal fungus *Glomus intraradices* is regulated in response to phosphate in the environment, MPMI, (volume 14), issue 10, pp. 1140-1148.

Marschner, H. (1995): Mineral nutrition of higher plants, 2nd. edition, Academic Press, San Diego, London.

Martin, C. A. and Stutz, J. C. (2004): Interactive effects of temperature and arbuscular mycorrhizal fungi on growth, P uptake and root respiration of *Capsicum annuum* L. Mycorrhiza (volume 14), pp. 241-244.

Maruyama-Nakashita, A.; Nakamura, Y.; Yamaya, T. and Takahashi, H. (2004): Regulation of high-affinity suphate transporters in plants: towards systematic analysis of sulphur signalling and regulation, Journal of Experimental Botany. (volume 55), issue 404, pp. 1843-1849.

Matt, P.; Geier, M.; Walch-Liu, P.; Engels, C.; Krapp, A. and Stitt, M. (2001): Elevated carbon dioxide increases nitrate uptake and nitrate redutase activity when tobacco is grown on nitrate, but increases ammonium uptake and inhibits nitrate reductase activity when tobacco is grown on ammonium nitrate, Plant, Cell and Environment (volume 24), issue 1119, pp. 1137.

Mau, J.-L.; Chen, C.-P. and Hsieh, P.-C. (2001): Antimicrobial effect of extractes from Chinese Chive, Cinnamon, and Corni Fructus, Journal of Agricultural Food Chemistry (volume 49), pp. 183-188.

McCallum, J.; Porter, N.; Searle, B.; Shaw, M.; Bettjeman, B. and McManus, M. (2005): Sulfur and nitrogen fertility affects flavour of field-grown onions, Plant and Soil (volume 269), pp. 151-158.

Miller, A. J. and Cramer, M. D. (2005): Root nitrogen acquisition and assimilation, Plant and Soil (volume 274), pp. 1-36.

Miyasaka, S. C.; Checkai, R. T.; Grunes, D. l. and Norvell, W. A. (1988): Methods for controlling pH in hydroponic culture of wintr wheat forage, Agronomy Journal (volume 80), pp. 213-220.

Moon, J.; Lee, H.; Kim, M. and Lee, I. (2005): Analysis of flowering pathway integrators in *Arabidopsis*, Plant Cell Physiology (volume 46), issue 2, pp. 292-299.

Moon, J.; Suh, S.-S.; Lee, H.; Choi, K.-R.; Hong, C. B.; Paek, N.-C.; Kim, S.-G. and Lee, I. (2003): The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*, The Plant Journal (volume 35), pp. 613-623.

Morgan, J. A. W.; Bending, G. D. and White, P. J. (2005): Biological costs and benefits to plant microbe interactions in the rhizosphere, Journal of Experimental Botany. (volume 56), issue 417, pp. 1729-1739.

Mortimer, P. E.; Archer, E. and Valentine, A. J. (2005): Mycorrhizal C costs and nutritional benefits in developing grapevines, Mycorrhiza (volume 15), pp. 159-165.

Nagy, R.; Karandashov, V.; Chague, V.; Kalinkevich, K.; Tamasloukht, M.; Xu, G.; Jakobsen, I.; Levy, A. A.; Amrhein, N. and Bucher, M. (2005): The characterisation of novel mycorrhiza-specific phosphate transporters from *Lycopersicon escelentum* and *Solanum tuberosum* uncovers functional redundancey in symbiotic phosphate transport in solanaceous species, The Plant Journal (volume 42), pp. 236-250.

Naturland (2006): Naturland Richtlinien Erzeugung, <http://www.naturland.de/downloads/richtlinien/nl_rl_erzeugung_01-2006.pdf> (18.05.06).

Neumann, E. and George, E. (2004): Colonization with the arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) enhanced phosphorus uptake from dry soil in *Sorghum bicolor* (L.), Plant and Soil (volume 261), pp. 245-255.

Nowak, J. (2004): Effects of arbuscular mycorrhizal fungi and organic fertilization on growth, flowering, nutrient uptake, photosynthesis and transpiration of geranium (*Pelargonium hortorum* L.H. Bailey `Tango Orange`), Symbiosis. (volume 37), pp. 259-266.

OECD (2002): OECD sheme for the application of international standard for fruit and vegetables Guidance on Objective Tests for Determining the Ripeness of Fruit (April 1998), <http://www.oecd.org/dataoecd/32/47/19515719.pdf> (18.05.06).

Oehl, F.; Sieverding, E.; Ineichen, K.; Ris, E.-A.; Boller, T. and Wiemken, A. (2005): Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems, New Phytologist (volume 165), pp. 273-283.

Oehl, F.; Sieverding, E.; Mäder, P.; Dubois, D.; Ineichen, K.; Boller, T. and Wiemken, A. (2004): Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi, Oecologia (volume 138), pp. 574-583.

Olsson, P. A.; Burleigh, S. H. and van Aarle, I. M. (2005a): The influence of external nitrogen on carbon allocation to *Glomus intraradices* in monoxenic arbuscular mycorrhiza, New Phytologist (volume 168), pp. 677-686.

Olsson, P. A.; van Aarle, I. M.; Gavito, M. E.; Bengtson, P. and Bengtsson, G. (2005b): ^{13}C incorporation into signature fatty acids as an assay for carbon allocation on arbuscular mycorrhiza, Applied and Environmental Microbiology (volume 71), issue 5, pp. 2592-2599.

Ortas, I. and Rowell, D. L. (2004): Effect of ammonium and nitrate on indigenous mycorrhizal infection, rhizosphere pH change, and phosphorus uptake by sorghum, Communications in Soil Science and Plant Analysis (volume 35), issue 13 & 14, pp. 1923-1944.

Owen, A. G. and Jones, D. L. (2001): Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant *N* acquisition, Soil Biology & Biochemistry (volume 33), pp. 651-657.

Ozores-Hampton, M.; Vavrina, C. S. and Obreza, T. A. (1999): Yard trimming-biosolids compost: possible alternative to sphagnum peat moss in tomato transplant production, Compost Science and Utilization (volume 7), issue 4, pp. 42-49.

Palenchar, P. M.; Kouranov, A.; Lejay, L. V. and Coruzzi, G. M. (2004): Genome-wide patterns of carbon and nitrogen regulation of gene expression validate the combined carbon and nitrogen (CN)-signaling hypothesis in plants, Genome Biology (volume 5), issue 11, pp. R91.

Pearson JN; Smith SE and Smith FA (1991): Effect of photon irradiance on the development and activity of VA mycorrhizal infection in *Allium porrum*, Mycological Research (volume 95), issue 6, pp. 741-746.

Peiwen, X.; Huisheng, S.; Ruijie, S. and Yuanjun, Y. (1994): Allium production and research in China, Acta Horticulturae (volume 358), pp. 127-131.

Perner, H.; Schwarz, D. and George, E. (2006): Effect of Mycorrhizal Inoculation and Compost Supply on Growth and Nutrient Uptake of Young Leek Plants Grown on Peat-based Substrates, HortScience. (volume 41), issue 3, pp. 628-632.

Peterson, R. L.; Massicotte, H. B. and Melville, L. H. (2004): Mycorrhizas: Anatomy and Cell Biology, 1. edition, CABI Publishing, Wallingford, Oxon.

Peuke, A. D.; Jeschke, W. D. and Hartung, W. (2002): Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation, Journal of Experimental Botany. (volume 53), issue 367, pp. 241-250.

Pinior, A.; Grunewaldt-Stöcker, G.; von Alten, H. and Strasser, R. J. (2005): Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll *a* fluorescence, proline content and visual scoring, Mycorrhiza (volume 15), pp. 596-605.

Pinior, A.; Wyss, U.; Piché, Y. and Vierheilig, H. (1999): Plants colonized by AM fungi regulate further root colonization by AM fungi through altered root exudation, Canadian Journal of Botany (volume 77), issue 6, pp. 891-897.

Piotrowski, J. S.; Denich, T.; Klironomos, J. N.; Graham, J. M. and Rillig, M. C. (2004): The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species, New Phytologist (volume 164), pp. 365-373.

Poulsen, K. H.; Nagy, R.; Gao, L.-L.; Smith, S. E.; Bucher, M.; Smith, F. A. and Jakobsen, I. (2005): Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus, New Phytologist (volume 168), pp. 445-454.

Poulton, J. L.; Bryla, D.; Koide, R. T. and Stephenson, A. G. (2002): Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato, New Phytologist (volume 154), pp. 255-264.

Raab, T. K. and Terry, N. (1995): Carbon, nitrogen, and nutrient interactions in beta-vulgaris L as influenced by nitrogen-source, NO_3- versus NH_4^+, Plant Physiology (volume 107), issue 2, pp. 575-584.

Raghothama, K. G. and Karthikeyan, A. S. (2005): Phosphate aquisition, Plant and Soil (volume 274), pp. 37-49.

Randle, W. M. (1992b): Onion germaplasm interacts with sulfur fertility for plant sulfur utilization and bulb pungency, Euphytica (volume 59), issue 2-3, pp. 151-156.

Randle, W. M. (1992a): Sulfur nutrition affects nonstructural water-soluble carbohydrates in onion germplasm, HortScience. (volume 27), issue 1, pp. 52-55.

Randle, W. M. and Bussard, M. L. (1993): Pungency and sugars of short-day onions as affected by sulfur nutrition, Journal of the American Society for Horticultural Science. (volume 118), issue 6, pp. 766-770.

Randle, W. M.; Lancaster, J. E.; Shaw, M. L.; Sutton, K. H.; Hay, R. L. and Bussard, M. L. (1995): Quantifying onion flavor compounds responding to sulfur fertility - sulfur increases levels of alk(en)yl cysteine sulfoxides and biosynthetic intermediates, Journal of the American Society for Horticultural Science. (volume 120), issue 6, pp. 1075-1081.

Rausch, C.; Daram, P.; Brunner, S.; Jansa, J.; Lalol, M.; Leggewie, G.; Amrhein, N. and Bucher, M. (2001): A phosphate transporter expressed in arbuscule-containing cells in potato, Nature. (volume 414), pp. 462-466.

Rausch, T. and Wachter, A. (2005): Sulfur metabolism: a versatile platform for launching defence operations, Trends in Plant Science (volume 10), issue 10, pp. 503-509.

Ravanel, S.; Gakiere, B.; Job, D. and Douce, R. (1998): Cystathionine g-synthase from *Arabidopsis thaliana:* purification and biochemical characterization of the recombinant enzyme overexpressed in *Escherichia coli*, Biochemistry Journal (volume 331), issue 639, pp. 648.

Raviv M; Zaidman B-Z and Kapulnik Y (1998): The use of compost as peat substitute for organic vegetable transplants production, Compost Science and Utilization (volume 6), issue 1, pp. 46-52.

Raviv, M.; Zaidman, B.-Z. and Kapulnik, Y. (1998): The use of compost as peat substitute for organic vegetable transplants production, Compost Science and Utilization (volume 6), issue 1, pp. 46-52.

Reddy, G. V.; Heisler, M. G.; Ehrhardt, D. W. and Meyerowitz, E. M. (2004): Real-time lineage analysis reveals oriented cell division associated with morphogenesis at the shoot apex of Arabidopsis thaliana, Development (volume 131), pp. 4225-4237.

Reddy, K. R. and Zhao, D. (2005): Interactive effects of elevated CO_2 and potassium deficiency on photosynthesis, growth, and biomass partitioning of cotton, Field Crop Research (volume 94), pp. 201-213.

Redecker, D. (2002): Palaeoglonius grayi from the Ordovician, Mycotaxon (volume 84), pp. 33-37.

Redecker, D. (2006): ISCB project, <http://plantbiology.unibas.ch/ecolmyc/ecolmyc.htm> (18.05.06).

Reinhardt, D.; Pesce, E.-R.; Stieger, P.; Mandel, T.; Baltensprenger, K.; Bennett, M.; Traas, J.; Frimi, J. and Kuhlemeier, C. (2003): Regulation of phyllotaxis by polar auxin transport, Nature. (volume 246), pp. 255-260.

Rillig, M. C.; Lutgen, E. R.; Ramsey, P. W.; Klironomos, J. N. and Gannon, J. E. (2005): Microbiota accompanying different arbuscular mycorrhizal fungal isolates influence soil aggregation, Pedobiologia (volume 49), pp. 251-259.

Rivera-Becerril, F.; Van Tuinen, D.; Martin-Laurent, F.; Metwally, A.; Dietz, K.-J. and Gianinazzi, S (2005): Molecular changes in Pisum L. roots during arbuscular mycorrhiza buffering of cadmium stress, Mycorrhiza (volume 16), pp. 51-60.

Roberts, J. K. M. and Pang, M. K. L. (1992): Estimation of ammonium ion distribution between cytoplasm and vacuole using nuclear-magnetic-resonance spectroscopy, Plant Physiology (volume 100), pp. 1571-1574.

Rubatzky, V. E. and Yamaguchi, M. (1997): World Vegetables: Principles, production and nutritive values, 2nd. edition, ITP, New York.

Russell, J. and Bulman, S. (2005): The liverwort Marchantia foliacea forms a specialized symbiosis with arbuscular mycorrhizal fungi in the genus Glomus, New Phytol (volume 165), issue 2, pp. 567-579.

Sáinz, M. J.; Taboada-Castro, M. T. and Vilarino, A. (1998): Growth, mineral nutrition and mycorrhizal colonization of red clover and cucumber plants grown in a soil amended with composted urban wastes, Plant and Soil (volume 205), pp. 85-92.

Saito, K. (2000): Regulation of sulfate transport and synthesis of sulfur-containing amino acids, Current Opinion in Plant Biology (volume 3), pp. 188-195.

Salerno, G. L. and Curatti, L. (2003): Origin of sucrose metabolism in higher plants: when, how and why?, Trends in Plant Science (volume 8), issue 2, pp. 63-69.

Sanders, F. E. (1975): The effect of foliar-applied phosphate on the mycorrhizal infections of onion roots, Sanders, F. E.; Mosse, B. and Tinker, P. B., Endomycorrhiza , pp. 261-276, Academic Press, London.

Sanders, F. E. and Tinker, P. B. (1971): Mechanism of absorption of phosphate from soil by *Endogone* mycorrhizas, Nature. (volume 233), pp. 278-279.

Scervino, J. M.; Ponce, M. A.; Erre-Bassells, R.; Vierheilig, H.; Ocampo, J. A. and Godeas, A. (2005): Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*., Mycological Research (volume 109), issue 7, pp. 789-794.

Schaller, K. (1988): Praktikum zur Bodenkunde und Pflanzenernährung, Geisenheimer Berichte 2. edition, Selbstverlag, Geisenheim, Germany.

Schilling, G. (2000): Pflanzenernährung und Düngung, UTB, Stuttgart.

Schjoerring, J. K.; Husted, S.; Mäck, G. and Mattsson, M. (2002): The regulation of ammonium translocation in plants, Journal of Experimental Botany. (volume 53), issue 370, pp. 883-890.

Schmidt, A. and Jäger, K. (1992): Open questions about sulfur metabolism in plants, Annual Review of Plant Physiology and Plant Molecualr Biology. (volume 43), pp. 325-349.

Schubert, A.; Allara, P. and Morte, A. (2003): Cleavage of sucrose in roots of soybean (Glycine max) colonized by an arbuscular mycorrhizal fungus, New Phytologist (volume 161), pp. 495-501.

Schüler, C.; Biala, J.; Bruns, C.; Gottschall, R.; Ahlers, S. and Vogtmann, H. (1989): Suppression of root rot on peas, beans and beetroots caused by *Phytium ultimum* and *Rhizoctonia solani* through the amendment of growth media with composted organic houshold waste, Journal of Phytopathology (volume 127), pp. 227-238.

Schüssler, A. (2006): *Glomeromycota* species list, <http://www.tu-darmstadt.de/fb/bio/bot/schuessler/amphylo/amphylo_species.html> (18.05.06).

Schwimmer, S. and Weston, W. J. (1961): Enzymatic Development of pyruvic acid in onion as a measure of pungency, Agriculture and Food Chemistry (volume 9), issue 4, pp. 301-304.

Shaul-Keinan, O.; Gadkar, V.; Ginzberg, I.; Grünzweig, J. M.; Chet, I.; Elad, Y.; Wininger, S.; Bclausov, E.; Eshed, Y.; Arzmon, N.; Ben-Tal, Y. and Kapulnik, Y. (2002): Hormone concentrations in tobacco roots change during arbuscular mycorrhizal colonization with *Glomus intraradices*, New Phytologist (volume 154), pp. 501-507.

Smith, F. W.; Ealing, P. M.; Hawkesford, M. J. and Clarkson, D. T. (1995): Plant members of a family of sulfate transporters reveal functional subtypes, Proceeding National Academic Society of the United States of America (volume 92), pp. 9373-9377.

Smith, F. W.; Hawkesford, M. J.; Ealing, P. M.; Clarkson, D. T.; Van den Berg, L. J. L.; Belcher, A. R. and Warrilow, A. G. S. (1997): Regulation of expression of a cDNA from barley roots encoding a high affinity sulphate transporter, The Plant Journal (volume 12), issue 4, pp. 875-884.

Smith, S. E. and Gianinazzi-Pearson, V. (1990): Phosphate Uptake and arbuscular activity in mycorrhizal Allium cepa L. : Effects of photon irradiation and phosphate nutrition, Aust.J.Plant Physiol. (volume 17), pp. 177-188.

Smith, S. E. and Read, D. J. (1997): Mycorrhizal Symbiosis, 2nd. edition, Academic Press, San Diego, California, USA.

Smith, S. E.; Smith, F. A. and Jakobsen, I. (2004): Functional diversity in arbuscular mycrrhizal (AM) symbioses: the contribution of the mycrrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake, New Phytologist (volume 162), pp. 511-524.

Son, C. L. and Smith, S. E. (1988): Mycorrhizal growth responses: interactions between photon irradiance and phosphorus nutrition, New Phytologist (volume 108), pp. 305-314.

Son, H.-J.; Park, G.-T.; Cha, M.-S. and Heo, M.-S. (2006): Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolate from soybean rhizosphere, Bioresource Technology (volume 97), pp. 204-210.

Steffens, D.; Stamm, R.; Yan, F.; Leithold, G. and Schubert, S. (1-3-0005): Rock phosphate mobilization by summer wheat, white lupin, and faba bean in a crop rotation, Kassel University Press GmbH Kassel, Germany.

Steffens, D.; Stamm, R.; Yan, F.; Leithold, G. and Schubert, S. (1-3-0005): Rock phosphate mobilization by summer wheat, white lupin, and faba bean in a crop rotation, Kassel Uuniversity Press GmbH Kassel, Germany.

Stitt, M.; Müller, C.; Matt, P.; Gibon, Y.; Carillo, P.; Morcuende, R.; Scheible, W.-R. and Krapp, A. (2002): Steps towards an integrated view of nitrogen metabolism, Journal of Experimental Botany. (volume 53), issue 370, pp. 959-970.

Stöppler-Zimmer, H.; Gottschall, R. and Gallenkemper, B. (1993): Anforderung an Qualität und Anwendung von Bio- und Grünkomposten, Arbeitskreis für Nutzbarmachung von Siedlungabfällen (volume 25), pp. 26-27.

Takahashi, H.; Yamazaki, M.; Sasakura, N.; Watanabe, A.; Leustek, T.; Engler, J. D.; Engler, G.; Van Montagu, M. and Saito, M. (1997): Regulation of sulfur assimilation in higher plants: A sulfate transporter induced in sulfate-starved roots plays a central role in *Arabidopsis thaliana*, Proceeding National Academic Society of the United States of America (volume 94), pp. 11102-11107.

Tamasloukht, M.; Sèjalon-Delmas, N.; Kluever, A.; Jauneau, A.; Roux, C.; Bécard, G. and Franken, P. (2003): Root factors induce mitochondrial-related gene expression and fungal respiration during the developmental switch from asymbiosis to presymbiosis in the arbuscular mycorrhizal fungus *Gigaspora rosea*, Plant Physiology (volume 131), pp. 1468-1478.

Tian, C. Y.; Feng, G. and Zhang, F. S. (2004): Different effects of arbuscular mycorrhizal fungal isolates from saline or non-saline soil on salinity tolerance of plants, Applied Soil Ecology (volume 26), pp. 143-148.

Toljander, J. F.; Artursson, V.; Paul, L. R.; Jansson, J. K. and Finlay, R. D. (2006): Attachment of different soil bacteria to arbuscular mycorrhizal fungal extraradical hyphae is determined by hyphal vitality and fungal species, FEMS (volume 254), pp. 34-40.

Tong, Y.; Zhou, J.-J.; Li, Z. and Miller, A. J. (2005): A two-component high-affinity nitrate uptake system in barley, The Plant Journal (volume 41), pp. 442-450.

Ullrich, C. I. and Novacky, A. J. (1990): Extra- and intracellular pH and membrane potential changes induced by K^+, Cl^-, $H_2PO_4^-$, and NO_3^- uptake and fusicoccin in root hairs of *Limnobium stoloniferum*, Plant Physiology (volume 94), pp. 1561-1567.

Urano, Y.; Manabe, T.; Noji, M. and Saito, K. (2000): Molecular cloning and functional characterization of cDNAs encoding cysteine synthase and serine acetyltransferase that may be responsible for high cellular cysteine content in *Allium tuberosum*, Gene (volume 257), pp. 269-277.

Usha, K.; Mathew, R. and Singh, B. (2005): Effect of three species of arbuscular mycorrhiza on bud sprout and ripening in grapevine (Vitis vinifera L.) cv. Perlette, Biological Argiculture and Horticulture (volume 23), pp. 73-83.

Valentine, A. J.; Osborn, B. A. and Mitchell, D. T. (2001): Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber, Scientia Horticulturae (volume 88), pp. 177-189.

Valli, G. and Giardina, E. G. V. (2002): Benefits, adverse effects and drug interactions of herbal therapies with cardiovascular effects, Journal of the American College of Cardiology. (volume 39), issue 7, pp. 1083-1095.

Van Beusichem, M. L.; Kirkby, E. A. and Baas, R. (1988): Influence of nitrate and ammonium nutrition on the uptake, assimilation, and distribution of nutrients in *Ricinus communis*, Plant Physiology (volume 86), pp. 914-921.

Van den Berg, L. J. L.; Dorland, E.; Vergeer, P.; Hart, M. A. C.; Bobbink, R. and Roelofs, J. G. M. (2005): Decline of acid-sensitive plant species in heathland can be attributed to ammonium toxicity in combination with low pH, New Phytologist (volume 166), issue 2, pp. 551-564.

Van Rhijn, P.; Fang, Y.; Galili, S.; Shaul, O.; Atzmon, N.; Wininger, S.; Eshed, Y.; Lum, M.; Li, Y.; To, V.; Fujishige, N.; Kapulnik, Y. and Hirsch, A. (1997): Expression of early nodulin genes in alfalfa mycorrhizae indicates that signal transduction pathways used in forming arbuscular mycorrhizae and Rhizobium-induced nodules may be conserved, Proceeding National Academic Society (volume 94), pp. 5467-5472.

Veeken, A.; de Wilde, V.; Woelders, H. and Hamelers, B. (2004): Advanced bioconversion of biowaste fpr production pf a peat substitute and renewable energy, Bioresource Technology (volume 92), pp. 121-131.

Vierheilig, H. (2004): Regulatory mechanism during the plant - arbuscular mycorrhizal fungus interaction, Canadian Journal of Botany (volume 82), pp. 1166-1176.

Villegas, J. and Fortin, J. A. (2001): Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH_4^+ as nitrogen source, Can.J.Bot (volume 79), issue 8, pp. 865-870.

Wagner, D.; Wellmer, F.; Dilks, K.; William, D.; Smith, M. R.; Kumar, P. P.; Richmann, J. L.; Greenland, A. J. and Meyerowitz, E. M. (2004): Floral induction in tissue culture: a system for the analysis of LEAFY-dependent gene regualtion, The Plant Journal (volume 39), pp. 273-282.

Walch-Liu, P.; Neumann, G.; Bangerth, F. and Engels, C. (2000): Rapid effects of nitrogen form on leaf morphogenesis in tobacco, Journal of Experimental Botany. (volume 51), issue 313, pp. 227-237.

Wang, H.; Parent, S.; Gosselin, A. and Desjardins, Y. (1993): Vesicular-arbuscular mycorrhizal peat-based substrates enhanced symbiosis establishment and growth of three micropropagated species, Journal of the American Society for Horticultural Science. (volume 118), issue 6, pp. 896-901.

Wang, R.; Guegler, K.; La Brie, S. T. and Crawford, N. M. (2000): Genomic analysis of a nutrient response in arabindopsis reveals divers expression patterns and novel metabolic and potential regulatory genes induced by nitrate, The Plant Cell (volume 12), pp. 1491-1509.

Wang, R.; Okomato, M.; Xing, X. and Crawford, N. M. (2003): Micorarry analysis of the nitrate response in arabidopsis roots and shoots reveals over 1,000 rapidly responding genes and new linkages to glucose, trehalose-6-phosphate, iron, and sulfate metabolism, Plant Physiology (volume 132), pp. 556-567.

Wang, Y.-H.; Gravin, D. F. and Kochian, L. V. (2001): Nitrate-induced genes in tomato roots. array analysis reveals novel genes that may play a role in nitrogen nutrition, Plant Physiology (volume 127), pp. 345-359.

Wells, A. T.; Chan, K. Y. and Cornish, P. S. (2000): Comparison of conventional and alternative vegetable farming systems on the properties of a yellow earth in New South Wales, Agriculture, Ecosystems and Environment (volume 80), pp. 47-60.

Whipps, J. M. (2004): Prospects and limitations for mycorrhizas in biocontrol of root pathogens, Canadian Journal of Botany (volume 82), issue 8, pp. 1198-1227.

Whitaker, J. R. (1976): Development of flavor, odor and pungency in onion and garlic, Advances in Food Research (volume 22), pp. 73-133.

White, P. J. (1996): The permeation of ammonium through a voltage-independent K^+ channel in the plasma membrane of rye roots, J.Membr Biol (volume 152), pp. 89-99.

Wills, R. B. H.; Wong, A. W. K.; Scriven, F. M. and Greenfield, H. (1984): Nutrient composition of chinese vegetables, Journal of Agriculture and Food Chemistry. (volume 32), issue 413, pp. 416.

Winter, H. and Huber, S. C. (2000): Regulation of sucrose metabolism in higher plants; localization and regulation of activity of key enzymes, Critical Reviews in Biotechnology and Molecular Biology (volume 35), pp. 253-289.

Yao, Q.; Li, X.; Ai, W. and Christie, P. (2003): Bi-directional transfer of phosphorus between red clover and perennial ryegrass via arbuscular mycorrhizal hyphal links , European Journal of Soil Biology (volume 39), pp. 47-54.

Yoo, K. S. and Pike, L. M. (2001): Determination of background pyruvic acid concentration in onions, *Allium* species, and other vegetables, Scientia Horticulturae (volume 89), pp. 249-256.

Yu, H.; Ito, T.; Peng, J.; Kumar, P. and Meyerowitz, E. M. (2004): Floral homeotic genes are targets of gibberellin signaling in flower development, PNAS (volume 101), issue 20, pp. 7827-7832.

Zhao, F. J.; Hawkesford, M. J. and McGrath, S. P. (1999): Sulphur assimilation and effects on yield and quality of wheat, Journal of Cereal Science (volume 30), pp. 1-17.

Zimmer, K. (1991): Hauptkulturen im Zierpflanzenbau, 3. edition, Ulmer Verlag, Stuttgart.

Acknowledgments

My first thanks go to my advisor, Prof. Dr. George, for the allocation of the topic of this thesis and the good working conditions. He was an exceptional supervisor who trained, supported and encouraged me gently at every point of my work. I thank him for his trust for doing my experiments self-dependent.

My thanks also go to Dr. Schwarz, my second supervisor. I benefited enormously from his experienced skills in experimental preparation and execution. I want to thank him for familiarizing me with the conventions in an institute out of university.

I am grateful to BMELV, DFG, and Sino-German Science Centre for financing part of this research work.

Many thanks to Eric Brewer for correcting the English of most of my work.

Sincere thanks are given to all colleagues of the IGZ and HU for establishing a pleasant working atmosphere and for their helpfullness. Specially mentioned be Gundula Aust for her encouragement. I also thank Mrs. Schmidt for elemental analysis; Andrea Maikath and Dr. Krumbein for assistance during the plant quality work; Dr. Kroschweksi for her advises in statistical analysis. Mr. Kunert, Mrs. Rathenow, Katrin Stefanowski, Hildegard Wohlfeil, Gisela Hasse, and Christa Henkel for horticultural assistance; and Mr. Hauschild for electrical management.

Special thanks go to the two students Juliane Makower and Jakob Witten for their untiring help with my experiments, probing questions, laughs and friendship.

I will always keep in warm memory the diploma, master, PhD students and post docs at the IGZ and HU, who contributed with their work to a lively scientific ambiance. I thank them for their sympathy and diverting company during long bus rides to work.

I also owe a huge gratitude to my parents who always supported me. Moreover, I thank Daniel Schreiber and all my friends that I can not mention individually for their friendship, motivation and them reminding me of a world besides the PhD.

Henrike Perner